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NEW EVIDENCE IN FAVOR OF A CHIEF VASOCONSTRICTOR CENTER IN THE BRAIN

STUDIES IN VASOMOTOR REFLEX ARCS. IV

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From the Anatomical Laboratory of Northwestern University Medical School

Received for publication September 30, 1916

Our knowledge of the anatomy of the vasomotor reflex arcs is still very imperfect. We believe that there exists in the brain stem a center for the regulation of blood pressure; but it is debated whether or not there is a separate vasodilator center, (1) and whether there is in addition a separate vasotonic center (2). The location of these centers and their relation to each other is unknown. In the cat faradic stimulation of the fovea inferior in the floor of the fourth ventricle produces a sharp rise in blood pressure; and stimulation of a point 3 mm. behind this in the area postrema causes an equally marked drop in pressure (3). But no evidence has been presented to show that these points mark the location of a vasoconstrictor and a vasodilator center respectively. Possibly they are only the termini of afferent pressor and afferent depressor fibers of the vagus, i.e., only parts of the afferent limbs of the corresponding reflex arcs.

The afferent impulses producing the pressor reflex from the sciatic nerve are transmitted through the spinal cord in the apex of the posterior gray column (4). When this was cut on both sides of the cord at the level of the first lumbar segment no pressor reflex could be obtained from stimulation of the sciatic nerve. The apex of the columna posterior is composed of the substantia gelatinosa Rolandi and the tractus dorsolateralis of Lissauer. It contains no long tracts but only short association fibers linking one segment of the cord with another. Any impulse ascending or descending along this path for more than a segment or two must travel over a series of many neurones.

When we found that the afferent limb of the pressor arc included this series of short fibers we were led to surmise that this reflex might be largely spinal. According to this conception the impulses, entering the cord by way of the dorsal roots associated with the sciatic nerve (fig. 1, *b*) would ascend by way of these short spinal fibers in the apex of the posterior horn (*e*) to the thoracic and upper lumbar segments of the spinal cord and then forward (*g*) to the efferent vasoconstrictor neurones of the white rami (*a*). That such a spinal reflex is possible has been demonstrated by Pike (5) and Sherrington (6), who showed that after section of the cervical cord and during cerebral anaemia the vascular tone and pressor reflexes, which at first were lost, returned again after a short time. Obviously they must then have been purely spinal. The fact that these reflexes are temporarily abolished on section of the cervical cord does not prove that the corresponding arcs pass through the medulla, because all spinal reflexes are temporarily abolished under the same conditions. Either we must accept the hypothesis of Pike that in the mammal there are no spinal reflexes or we must admit that spinal shock (a disturbance of nervous equilibrium which von Monakow has called "diaschisis," Herrick (7)) may temporarily abolish the function of purely spinal arcs. If we make the latter admission it would apply as well to the vasomotor as to the other reflexes and we would be without evidence justifying us in locating the vasomotor reflex centers in the medulla. When analyzed in this way the conception of a chief vasoconstrictor center in the mnsdulla is seen to rest on rather insecure grounds.

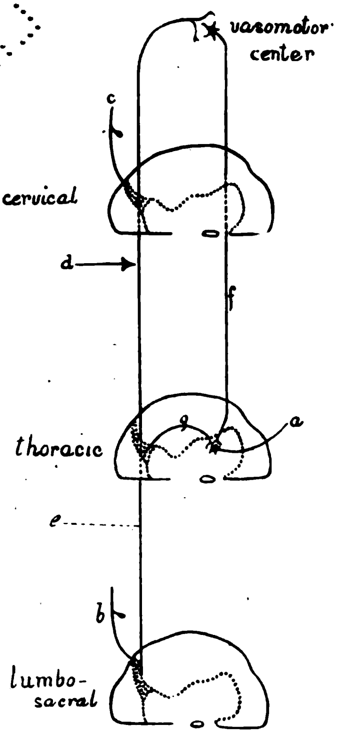

Fig. 1. Diagram to illustrate the paths which might be followed by vasomotor impulses: *a*, efferent fiber of a white ramus; *b* and *c*, afferent fibers of dorsal roots; *d*, level of a lesion in the posterior gray columns; *e*, line indicating the path of afferent pressor impulses in the posterior gray column.

Any experiment designed to test this conception must be so carried out that spinal shock or "diaschisis" is not produced. In other words the skeletal reflexes must not be affected by the conditions of the experiment. It is with evidence of this kind that the present paper deals. The apex of the posterior gray column, including the dorsalateral tract of Lissauer and the substantia gelatinosa Rolandi, was cut on both sides of the spinal cord at the level of the first thoracic segment. This interrupts the pathway for afferent pressor impulses at the level of the arrow, *d*, figure 1. This is above the level of the thoracico-lumbar autonomic outflow through the white rami (*a*) but below the origin of the brachial nerves (*c*). If the pressor arc were purely spinal the sciatic reflex would not be affected by this lesion. The impulses from the sciatic entering along the unmyelinated fibers of the dorsal roots (*b*) would ascend in the apex of the posterior horn (*e*) to the thoracic and upper lumbar segments of the spinal cord and then pass forward through the gray matter (*g*) and out through the white rami (*a*). The lesion at *d* would however interrupt a hypothetical pressor path from the brachial nerves (*c*) down the apex of the posterior gray column to the thoracico-lumbar autonomic system (through *g* to *a*). In other words if the pressor reflex were purely spinal the lesion in question should not affect the reaction from the sciatic nerve but should abolish the reflex from the brachial nerves. But if the reflex were bulbar and the afferent limb of the arc followed the apex of the posterior column through the spinal cord to the medulla, the lesion would interrupt the arc from the sciatic nerve but not that from the brachial. This experiment gives us an opportunity to determine whether the pressor reflex is under normal conditions purely spinal or to what extent a bulbar chief vasoconstrictor center is involved.

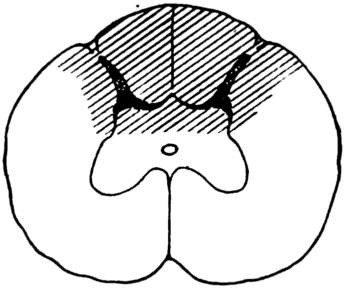

Fig. 2. Diagram showing the extent of the lesion in the second thoracic segment of the spinal cord of cat B10.

TECHNIQUE

Adult cats were used for these experiments. The spinal cord was exposed at the level of the second thoracic segment under rigid aseptic precautions. Since it had been previously shown that the posterior funiculi were not concerned in any of the vasomotor reactions an extensive lesion was made involving both posterior gray columns and both posterior funiculi (fig. 2). This operation is simpler and more certain than that of cutting the apices of the posterior columns alone and answers the purpose of the present experiment just as well. The animals recovered promptly and showed no signs of paralysis. They were tested at intervals for sensibility to pain. The technique throughout was the same as in the earlier experiments from the account of which we quote (4):

> After complete recovery of the animal from the lesion and after the conduction of pain in the cord had been tested the vasomotor reflexes were studied. Under ether anaesthesia a tracheotomy was performed and the ether bottle attached. Connections were made as rapidly as possible for carotid blood pressure and respiratory tracings. Both sciatic nerves were exposed, ligated and cut distally to the ligature. The central end was laid bare for some distance above the ligature and could be handled by the attached thread. On the left side three brachial nerves, viz.: the median, ulnar, and internal cutaneous, were exposed, ligated together, and cut distally to the ligature and thereafter treated as a single nerve. Care was taken not to stretch the nerves at any time. When not being stimulated, they were kept covered with other tissues to prevent drying.
>
> To eliminate passive dilation of blood vessels in the areas supplied by the divided nerves the limbs were constricted with heavy cord placed proximal to the elbow and knee joints. Fluctuations in blood pressure from pressure on the abdomen by flexion of the limbs during stimulation of a nerve were prevented by securely tying the legs to the animal board.
>
> The stage of ether anaesthesia is of the greatest importance. The animal was kept relaxed, with regular respiration, with pupils half contracted and with a brisk corneal reflex. Any tendency toward cyanosis was prevented.
>
> Faradic stimulation of the central ends of the cut nerves was used to elicit the reflexes. Standard platinum electrodes were applied at least half an inch from the cut ends of the nerves, held suspended by the threads with which they had been ligated. The electrodes were moved slowly along the nerves during stimulation. The source of the current was a Stoelting inductorium No. 7090 through the primary of which passed a constant half ampere current.
>
> Curare was used in some of the experiments.
>
> After the reflex vasomotor and respiratory responses had been recorded the animal was killed. A stretch of cord about 7 mm. long containing the lesion was removed and prepared by the pyridine-silver method and cut into serial sections. The serial sections were then studied to determine the extent of the lesion.

After the operations the animals were allowed to live for periods varying from four to thirty-six days before the vasomotor reflexes were tested.

RESULTS

We will discuss our observations on pain and the depressor reflexes in these cats in a subsequent paper and confine our attention here to a comparison of the pressor reflexes obtained from the brachial and the sciatic nerves. That it is reasonable to compare the results of stimulation of the brachial and sciatic nerves is shown by the fact that in normal cats stimulation of these nerves gives equivalent results. This statement is based not only on our own experience but also on that of Porter and Richardson (8), who found by a long series of experiments with stimuli of given intensity, applied to the sciatic and brachial nerves in cats and other animals, that the result did not depend in any way on which afferent spinal nerve was stimulated. According to Vincent and Cameron (9) all sensory spinal nerves "yield similar reflexes if sufficient care be taken to develop in all cases an equivalent stimulation of a roughly equal number of fibers." In our work we have used the three brachial nerves held together by a common ligature. These have a bulk roughly equal to that of the sciatic. We feel that much safer conclusions can be drawn by comparing the results of stimulation of the two in the same experiment than by a comparison of the results from the same nerve in normal and injured animals.

TABLE

Vasomotor reactions obtained from cats in which the posterior funiculi and posterior gray columns had been divided at the level of the second thoracic segment from 4 to 35 days previously. Faradic stimulation, secondary coil at 5. Rise or fall indicated in millimeters of mercury

CAT NO.	LESION		RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVES
	Extent	Duration			
		days			
B7	Complete	4	8	5	32
B9	Complete	6	28	30	46
B10	Complete	16	0	0	28
B11	Complete	18	0	0	18
B12	Incomplete	15	18	10	26
B14	Complete	30	− 6	− 8	14
B15	Complete	36	−26	−14	28
B16	Complete	35	16	0	40

We have for comparison the results obtained under similar conditions from stimulation of the normal cat's sciatic. Table 1 of a previous paper (4) shows that strong stimulation of the sciatic in 14 normal cats gave a rise in blood pressure in all but 2. The position of the secondary coil varied from 4 to 6. The rise obtained varied from 8 to 48 mm. Hg.

In the present series of experiments a good pressor reflex was obtained in each case from strong faradic stimulation of the brachial nerves with the secondary coil at 5. The rise in blood pressure varied from 14 to 46 mm. as indicated in the table. Stimulation of the sciatic with the same strength of current gave no response or a slight fall in four of the eight experiments. In three other cases a slight pressor response was obtained from one or both sciatics. In one cat a good rise was obtained from both sciatics, but even in this case the reaction obtained from the brachial nerves was considerably greater.

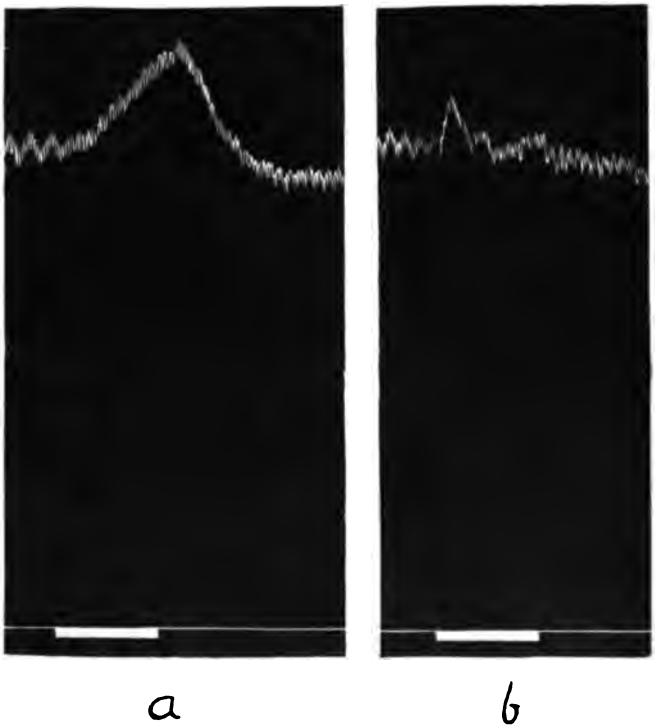

Fig. 3. Carotid blood pressure tracing from cat B10. Faradic stimulation, secondary coil at 5 of (*a*) three brachial nerves together and (*b*) the left sciatic nerve.

Figure 3 shows the character of the reactions obtained from cat B 10, with cord lesion at the level of the second thoracic segment the extent of which is indicated in figure 2. The brachial nerves (*a*) gave a rise in blood pressure of 28 mm. The left sciatic (*b*) gave no vasomotor reaction, but there were strong muscular contractions and these are indicated in the figure by the sharp irregularities of the tracing. The sciatic reaction was taken immediately after the brachial and the depth of anaesthesia and other conditions were the same. Such muscle spasms are frequently produced by stimulation of the sciatic in cats with lesions involving the posterior gray columns, when no spasms are produced by similar stimulation of the brachial nerves. We will discuss this observation in another paper.

Remembering that in normal cats the brachial and sciatic nerves give the same reactions, it is clear that in this series of experiments the lesion has more or less completely interrupted the pressor reflex arc from the sciatic without affecting that from the brachial nerves. This shows that the afferent pressor impulses from the sciatic travel up the cord to a vasoconstrictor center in the brain. That is to say the pressor reflex arc is not complete within the spinal cord. The path indicated by *g* in figure 1, carrying impulses from the apex of the posterior gray columns to the efferent vasomotor neurones, must have played a minor rôle, if any, in the reactions here recorded.

It is also obvious that the pathway from the vasoconstrictor center to the efferent vasomotor neurones must be located either in the ventral or lateral funiculi since it was injured in none of these experiments. This path is a part of the intact pressor reflex arc from the brachial nerves through the bulbar vasomotor center to the efferent vasomotor neurones of the white rami.

It is not possible at present to give a satisfactory interpretation of those cases in which more or less of a pressor reflex was obtained from the sciatic nerve. It may be that in these cases the pressor impulses passed forward along the path indicated by *g* in figure 1. We know that it is possible for the impulses to take this course after transection of the cervical cord. But before it could be said that this path functioned in these cases it would be necessary to be sure that the lesion had interrupted the only possible pathway for pressor impulses from the sciatic to the bulbar vasomotor center.

CONCLUSIONS

1. Pressor reflexes from the spinal nerves normally involve a bulbar vasoconstrictor center.

2. The afferent limb of the pressor arc from the sciatic nerve follows the apex of the posterior horn up to the level of the second thoracic segment and probably throughout the cord.

3. The efferent limb of the pressor arc lies in the lateral or ventral funiculi.

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AFFERENT SPINAL PATH FOR THE DEPRESSOR REFLEX

STUDIES IN VASOMOTOR REFLEX ARCS. V

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In the first paper (1) of this series it was demonstrated that the afferent impulses producing the pressor reflex travel along the cord in the apex of the posterior gray column. By a process of exclusion it was also shown that those responsible for the depressor reflex must be conducted by either the ventral or lateral funiculi. We were unable to secure a positive demonstration of this by the technique used in the former experiments because all those cats in which the ventral part of the lateral funiculi were cut developed paralysis and anaesthesia of the hind limbs and incontinence of urine. These symptoms did not appear in our other experiments and we are at a loss to account for them in these. However it seems not unlikely that hemorrhage from the ventrally placed extradural venous plexus may have resulted in a hematoma large enough to have produced pressure on the cord. In order to avoid this difficulty we abandoned the habit of letting the animals recover from the lesion before making the vasomotor tests, and carried out the operation and tests in one-half day.

TECHNIQUE

Under ether anaesthesia and without aseptic precautions the spinal cord was exposed in the region of the first lumbar segment. In working on the lateral funiculus it is necessary to prevent the knife from going through the dura into the extra dural venous plexus in the ventrolateral part of the canal. After the dura had been opened by a median longitudinal incision one tooth of the ligamentum denticulatum was cut and a strip of celluloid inserted between the cord and the dura in the interval between the fila of the first and second lumbar nerve roots. The knife was then passed through the lateral funiculus with its sharp edge directed outwards and the cut was completed by pressing the edge against the celluloid plate. This procedure was then repeated on the opposite side. Two of the nine cats were allowed to come out from under the anaesthetic and their hind limbs were tested for sensibility to pain after which they were again anaesthetized and a record made of the vasomotor and respiratory responses to stimulation of the brachial and sciatic nerves. In the other eight cats the operation was done and the records obtained without interrupting the anaesthetic.

The experiment was followed by an autopsy and a microscopic study of serial sections of the portion of the cord containing the lesions. In this way the location and extent of the lesion was accurately determined.

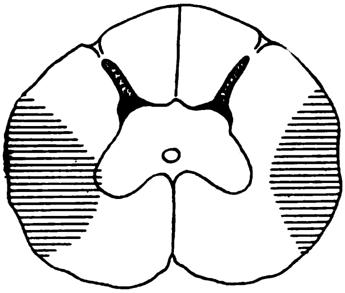

Fig. 1. Diagram of the first lumbar segment of the spinal cord of cat 91, showing the extent of the lesions in the lateral funiculi.

In testing the pain sense, pricking and pinching the skin and electrical stimulation through cutaneous electrodes were used as stimuli. A sharp cry and struggling were taken as evidences that pain was felt.

The technique of testing the vasomotor reflexes was the same as that given in the preceding paper on page 4 of this issue.

The autopsies showed that the lesion was in every case at the level of the first lumbar segment. The extent of the lesion as seen in microscopic sections varied considerably in the different cords but the lesions in cat 91, represented in figure 1, may be taken as typical. In some the injury was more extensive and in others less extensive than in this case.

RESULTS

When weak faradic stimulation was applied to the brachial nerves a drop in blood pressure was obtained in every case. As may be seen by reference to the table this drop varied in extent, the least being 5 and the greatest 32. These reactions represent typical depressor reflexes and the variation is no greater than in the reactions obtained from normal cats. They show that the vasomotor mechanism for depressor reflexes has not been impaired by the shock and hemorrhage incident to the preparation of the animal for experiment.

In sharp contrast to the reactions from the brachial nerves, are those from the sciatics. The same weak stimulation which consistently gave a drop when applied to the brachial nerves usually produced no effect when applied to the sciatics, and when there was a change in blood pressure it was as often a rise as a fall. The details are given in the table. The character of the reaction is shown in figure 2. It is evident that the lesions indicated in figure 1 interrupted the spinal afferent path for the depressor reflex from the sciatic nerves, figure 2, *b*.

But the lesions in the lateral funiculi did not interfere with the pressor reflex. On the contrary with faradic stimulation of medium intensity (secondary coil at 9 or 7) considerably better pressor reflexes were obtained from the sciatics than from the brachial nerves. Figure 3 shows that faradic stimulation with secondary coil at 9 produced no response from the brachial nerves, but shortly afterward the same stimulation applied to the left sciatic nerve gave a considerable rise in blood pressure. With stronger stimuli (secondary coil at 5) there was no constant difference between the reactions obtained from the brachial and sciatic nerves.

Table showing reflex changes in blood pressure after partial section of both lateral funiculi. Weak faradic stimulation, secondary coil at 15. Rise and fall indicated by + and − respectively and expressed in millimeters of mercury

CAT NO.	RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL
86	+4	+4	−32
90	−3	0	−10
91	0	0	−20
92	0	−2	− 8
93	0	0	− 8
94	−8	0	−16
99	0	0	−14
100	0	0	−10
101	+15	+10	− 5

These experiments seem to give additional evidence in favor of a balanced action between the pressor and depressor reflexes. In a former paper (1) the following theory was formulated:

> In the vasomotor reflexes there are two separate paths from the sciatic nerve to the efferent vasomotor neurones and for brevity we will speak of a pressor path and a depressor path according to the vascular responses produced by the impulses which they carry. The impulses traveling along the two paths are

mutually antagonistic and in normal animals vasomotor reactions represent a balance between them. The depressor path has a low threshold, and by it weak stimuli are able to reach the efferent vasomotor neurones. The pressor path has a high threshold since it is composed of the short fibers and many synapses of the apex of the posterior horn. With weak stimuli the impulses pass up the depressor path only, resulting in a drop in blood pressure. With stimuli of medium intensity they pass up both paths but the strong pressor overcome the weaker depressor impulses, resulting in increased blood pressure.

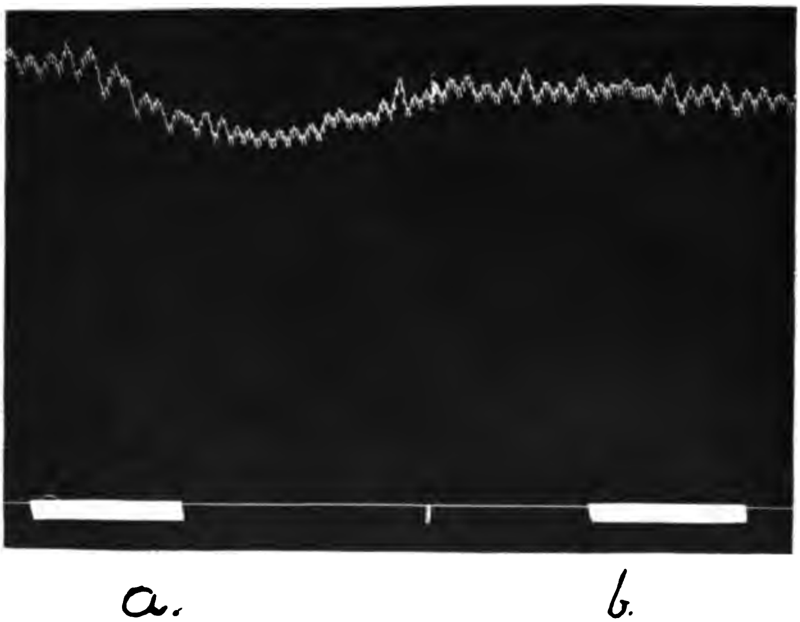

Fig. 2. Carotid blood pressure tracing from cat 91. Weak faradic stimulation (secondary coil at *15*) of the brachial nerves, *a*, and the left sciatic nerve, *b*. The drum was stopped at the point marked on the base line.

This theory is supported by the results of the present series of experiments. When the depressor path in the lateral funiculus has been divided, the pressor impulses arising from stimuli of medium intensity are able to manifest themselves in a rise in blood pressure, because they are no longer counterbalanced by the depressor impulses. In fact it would be difficult to account in any other way for the fact that, after section of the lateral funiculi in the region of the first lumbar segment, stimuli of medium intensity give better pressor reactions from the sciatic than from the brachial nerves.

There are two ways in which this balanced action might take place: 1. It may be a matter of inhibition, the depressor impulses inhibiting the vasoconstrictor center and the pressor impulses inhibiting the vasodilator center. 2. It may represent only the algebraic sum on blood pressure of the vasoconstriction and vasodilation resulting from the antagonistic afferent impulses.

Martin and Mendenhall (2) who have recently formulated a somewhat similar conception of the relation of the depressor to the pressor reflexes believe that the balance is in the form of the algebraic summation of the two actions rather than in the form of a mutual inhibition of the two centers.

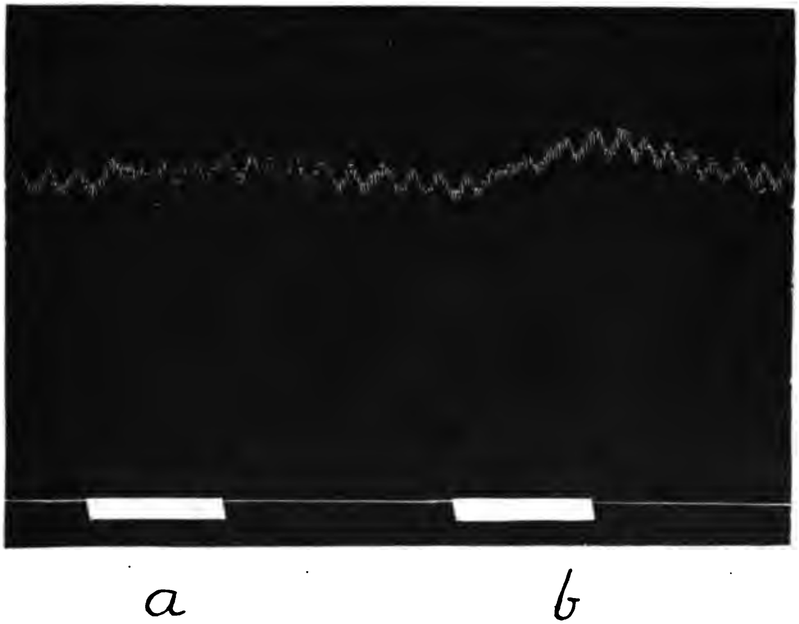

Fig. 3. Carotid blood pressure tracing from cat 91. Faradic stimulation of medium strength (secondary coil at *9*) of the brachial nerves, *a*, and the left sciatic nerve, *b*.

The fact that after the section of the lateral funiculi at the level of the first lumbar segment the pressor reflexes from the sciatic are as good or better than those from the brachial, is important evidence in favor of the specific character of the results obtained by the division of the posterior gray columns in our other experiments. Against all these other experiments the objection might well have been raised that there was perhaps nothing specific in the results. They might be explained on the assumption that strong impulses must reach the vasomotor center to produce a rise in blood pressure, and any extensive cord lesion involving afferent paths might so reduce the bulk of the impulses reaching this center as to weaken or obliterate the rise. We had shown, it is true, that the long fibers of the posterior funiculi had nothing to do with the vasomotor reactions. The present set of experiments completes the proof that the afferent impulses for the pressor and depressor reflexes follow separate paths in the spinal cord. The pressor path in the posterior gray columns can be interrupted without interfering with the depressor reflex; and the depressor path in the ventral funiculus can be interrupted without interfering with the pressor reflex.

A glance at figure 1 at once suggests the idea that the depressor path occupies much the same position in the cord as the spinothalamic tract. It occurred to us that the two might be identical, and in this case the afferent impulses would pass to the thalamus and then down to the vasomotor center or centers, and the reflex should be obliterated by decerebration. The depressor reflexes in decerebrate animals do not seem to have been thoroughly investigated by any one. In the earliest studies of these reflexes decerebration seems to have been one of the standard methods of procedure and, also, in at least one recent study, by Martin and Mendenhall (2), it was employed. This clearly indicates that the depressor reflexes are not obliterated by decerebration. This conclusion has been verified for us by Mr. W. N. Rowley of the Physiology Department. He performed decerebration on a number of dogs and found that the depressor reflexes were present and perhaps somewhat exaggerated. We wish to express our appreciation of his help in making this point perfectly clear.

On the basis of Mr. Rowley's work we may say with certainty that the depressor path is not the same as the spinothalamic tract although it occupies the same position in the spinal cord.

In the two cats which were allowed to come out from under the anaesthetic between the operation and the vasomotor tests no hypalgesia of the hind limbs could be demonstrated.

CONCLUSIONS

1. The afferent spinal path for depressor reflexes lies in the lateral funiculus in the same position as the spinothalamic tract, but it is composed of fibers that do not ascend above the rhombencephalon.
2. Section of this part of both lateral funiculi at the level of the first lumbar segment obliterates the depressor reflex from the sciatic nerves. The pressor reflex from stimuli of medium intensity applied to the sciatic nerves appears to be augmented.

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AFFERENT SPINAL PATHS AND THE VASOMOTOR REFLEXES

Studies in Vasomotor Reflex Arcs. VI

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There are many problems connected with the vasomotor reflexes which are still obscure. One of the most fundamental of these is the question: Why does the stimulation of a sensory nerve cause a rise in blood pressure under certain conditions and a drop under other conditions? It has long been known that stimulation of the depressor nerve gives only a drop in blood pressure, and that under the usual conditions stimulation of afferent spinal nerves gives only a rise. But, as was shown by Hunt (1) under carefully controlled conditions almost any afferent nerve can be made to give a depressor reflex. When a nerve is chilled the same stimulus, which before gave a rise, will cause a drop in pressure. It was known by Hunt and has been recently emphasized by Porter and others (2) that very weak stimuli will quite uniformally give depressor reflexes from any afferent nerve, except perhaps the splanchnic, and that strong stimulation of any afferent nerve, except the depressor nerve, will give a pressor reflex. It has been quite generally assumed that these facts could best be explained by the assumption that there are two kinds of afferent vasomotor fibers which have been called pressor and depressor fibers respectively. We propose to examine this old hypothesis in the light of our present knowledge of the afferent nervous system and of the vasomotor reflexes.

Very recently Vincent and Cameron (3) have opened up again the whole question of the significance of the depressor reflexes by some very suggestive observations: namely, that when an animal is under a certain light degree of ether anaesthesia, stimulation of afferent nerves produces very rapid deep respirations which in themselves produce a drop in blood pressure. Opening the thorax or giving curare does away with this respiratory effect and converts the drop into a rise. They state that they have not been able to observe a fall in blood pressure from weak stimulation nor from the stimulation of a nerve that has been cooled when the thorax has previously been opened. This is calculated to throw doubt on the existence of a true depressor reflex from the spinal nerves. We shall refer to these observations again.

We have shown that there are in the spinal cord two separate paths to the vasomotor centers—one for pressor and the other for depressor impulses. It is the purpose of the present paper to present additional evidence along this line, to summarize that which has been presented in other papers of this series and to consider the relation of these observations to the general theory of the vasomotor reflexes.

The object of the experiments, which are to be reported here, was to determine whether the effect of a lesion in the pressor path was dependent on the level of the cord in which it was located. This investigation was suggested by the difference in the reflexes obtained from cats in which the pressor path had been destroyed at the level of the first lumbar segment (4) and those in which it had been destroyed at the level of the second thoracic (5). Accordingly three additional series of experiments were made in which the apices of the posterior horns were divided at the level of the third lumbar segment in six cats, at the level of the first lumbar segment in seven cats, and at the level of the ninth thoracic segment in four cats. In order to determine whether the conduction was homolateral or contralateral the apex of the posterior horn was divided only on the right side in the first lumbar segment of six other cats.

TECHNIQUE

The procedure was the same as that followed in the preceding investigations. The operations were aseptically performed according to the technique detailed in the first paper of this series (4). The cats recovered rapidly from the effects of the operation. They were allowed to live for varying periods and were with few exceptions in first class condition at the time of the experiment. One showed some slight paralysis of the hind limbs and two others were sick with an infectious disease of the respiratory tract. Repeatedly, at varying periods after the operation, the animals were tested for alterations of the pain sense in the hind limbs. Finally a thorough examination was made of the vasomotor reflexes. The technique of testing the pain sense and making the tracing of the vasomotor reflexes was the same as that used in the preceding investigations. (4), (5). In each case an autopsy was performed, the lesion was accurately located and its extent determined by a study of serial sections.

Throughout these experiments the reactions obtained from the brachial nerves, situated above the lesion, were used as controls for the reactions from the sciatic. In another place (5) we have shown that in a normal animal identical reactions are obtained from the brachial and sciatic nerves, and that more reliable results can be obtained in this way than by comparing the sciatics of normal and injured animals.

BILATERAL LESIONS IN THE APICES OF THE POSTERIOR GRAY COLUMNS

The apices of both posterior gray columns were divided in twenty-five cats, in eight at the level of the second thoracic, in four at the level of the ninth thoracic, in seven at the level of the first lumbar, and in six at the level of the third lumbar segment. In five of these the vasomotor reactions were atypical since a rise in pressure was obtained from very weak stimulation. These atypical results are recorded by themselves in table 2. The reactions from the other twenty cats are given in table 1.

The first division of table 1 represents the vasomotor reactions obtained from cats in which the apices of both posterior gray columns had been divided along with the posterior funiculi at the level of the second thoracic segment. Note that with strong faradic stimulation (secondary coil at 5) good pressor reflexes were obtained from the brachial nerves in each cat, while from the sciatic nerves a moderate rise was obtained in three, no reaction in two, and a drop in two cats. It is obvious that the pressor reflex from the sciatic was decreased in every case, although in three cats it was not entirely obliterated. In presenting these results in another place (5), the question was raised as to whether the pressor reflexes obtained from the sciatics in these cats were purely spinal, i.e., whether there was in addition to the path by way of the medulla oblongata, also an alternative purely spinal arc for these reflexes which was complete below the second thoracic segment. We have introduced these results again

TABLE 1

Reflex changes in blood pressure after section of the apices of the posterior gray columns at different levels. Strong faradic stimulation, secondary coil at 5. Rise or fall in millimeters of mercury

CAT NO.	LESION			RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVE
	Level	Extent	Duration			
B7	II T.	Complete	4 days	+ 8	+ 5	+32
B9	II T.	Complete	6 days	+28	+30	+46
B10	II T.	Complete	16 days	0	0	+28
B11	II T.	Complete	18 days	0	0	+18
B14	II T.	Complete	30 days	− 6	− 8	+14
B15	II T.	Complete	36 days	−26	−14	+28
B16	II T.	Complete	35 days	+16	0	+40
80	IX T.	Incomplete	30 days	− 5	− 5	0
81	IX T.	Incomplete	29 days	+ 5	+12	+20
82	IX T.	Incomplete	26 days	−20	−16	+30
83	IX T.	Complete	26 days	−14	− 6	+16
B1	I L.	Complete	3 hours	−12	+10	+40
B2	I L.	Complete	1 day	−30	−34	−28
B4	I L.	Complete	24 days	0	0	+36
76	I L.	Complete	13 days	−26	−14	+44
77	I L.	Incomplete	12 days	−20	+20	+40
68	III L.	Complete	29 days	− 3	− 3	+12
69	III L.	Complete	30 days	−10	− 8	+20
71	III L.	Complete	30 days	−25	−30	+30
72	III L.	Complete	25 days	−30	−28	+28

TABLE 2

Atypical vasomotor reflexes obtained from cats in which very weak stimulation gave a rise in blood pressure. The apices of the posterior gray columns had been divided. Strong faradic stimulation, secondary coil at 5. Rise in millimeters of mercury

CAT NO.	LESION			RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVES
	Level	Extent	Duration			
B12	II T.	Incomplete	15 days	18	10	26
78	I L.	Complete	14 days	20	30	40
79	I L.	Incomplete	14 days	4	8	16
67	III L.	Complete	34 days	22	20	56
70	III L.	Complete	30 days	30	10	50

in this place in order to compare them with those obtained from cats with similar lesions at other levels.

The second division of table 1 gives the results obtained from cats in which the apices of both posterior horns had been divided at the level of the ninth thoracic segment. With the exception of one case, in which the lesion was incomplete, strong faradic stimulation (secondary coil at 5) gave a drop instead of a rise out of the sciatic nerves.

The third division of table 1 gives the results obtained from cats in which the apices of both posterior horns were divided at the level of the first lumbar segment. The lesions in cat 76 were typical and their position and extent is shown diagrammatically in figure 1. With strong faradic stimulation (secondary coil at 5) a drop was usually obtained from the sciatic nerves and a rise from the brachial nerves (see figure 2 and the table). In two of these cats a drop was obtained from the right sciatic and a rise from the left, but in one of the two the lesion was incomplete. The other cats gave either no reaction or a drop from strong stimulation of the sciatic.

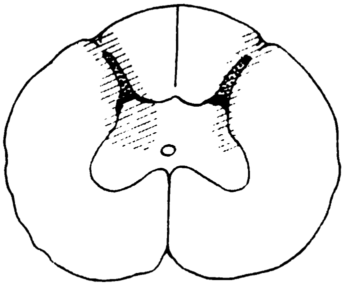

Fig. 1. Diagram of the first lumbar segment of cat 76 showing the extent of the lesions in the posterior gray columns.

The fourth division of table 1 gives the results obtained from cats in which the apices of the posterior horns had been divided at the level of the third lumbar segment. In each case strong stimulation gave a rise in blood pressure from the brachial and a drop from the sciatic nerves.

The last three divisions of the table show a pressor reflex only once from the sciatic when the lesion was complete and in this one cat the left sciatic only gave a rise while the right gave a drop. In one other cat with a complete lesion, B. 4, no vasomotor reflexes were obtained from the sciatics. In all the others with complete lesions strong stimulation of the sciatic gave a drop instead of a rise. Comparing these results with those indicated in the first division of this table it will be seen that there a rise is more frequent and a drop less frequent than in the last three divisions.

We believe that these differences are significant and that a lesion involving the posterior gray columns at the level of the second thoracic segment produces less disturbance of the pressor reflex from the sciatic than lesions in this column lower down, because in the case of the higher lesion there is open an alternative path forward through the gray matter to the efferent vasomotor neurones. While impulses traveling this path are unable to develop the full pressor reflex as indicated by the reaction from the brachial nerves in the same animal, they are usually able either to produce a slight rise or to balance the depressor impulses, producing a negative result. This is what one should expect since it is known that such a purely spinal path exists after transection of the cervical cord (6).

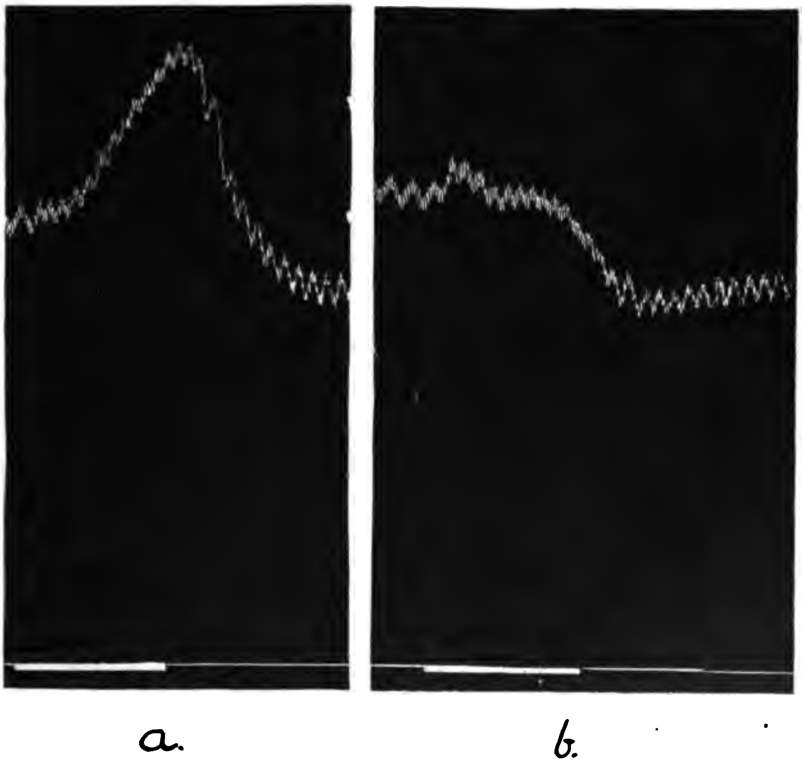

Fig. 2. Carotid blood pressure tracing from cat 76. Strong faradic stimulation, secondary coil at 5. *a*, brachial nerves; *b*, right sciatic nerve.

The last three divisions of the table serve to emphasize in a striking way the importance of the apices of the posterior gray columns for the conduction of the afferent pressor impulses in the spinal cord.

In five other cats, represented in table 2, the vasomotor reactions were atypical in that with very weak stimuli (secondary coil at 15) a rise in blood pressure was obtained. In all our other experiments stimuli of this strength have either given a fall or no change in pressure. In these five cats the lesion in the posterior gray column did not obliterate the pressor reflex from the sciatic nerves. It is obvious because of the weak stimuli with which they could be produced that some factor entered into these reactions, which is not present in the typical pressor reflex. Just what this factor is we are unable to say. Cannon and Hoskins (7) have shown that stimulation of the sciatic nerve causes the discharge into the blood of appreciable quantities of adrenin and this is probably not without effect on the blood pressure. The rise in pressure in our atypical cats occurred rather too promptly to be accounted for in this way. We bring up the matter of adrenin discharge not so much as an explanation as to show how diverse are the factors which enter into these reactions.

UNILATERAL LESION IN THE APEX OF THE RIGHT POSTERIOR GRAY COLUMN

It was shown in a former paper that after section of the right half of the spinal cord the pressor reflex from both sciatics was reduced, that from the right more so than that from the left. From these facts it was concluded "that the afferent impulses producing a rise in blood pressure are conducted bilaterally in the cord, and equally well on both sides or somewhat better homolaterally." In order to determine whether the pressor impulses travelled chiefly through the homolateral posterior gray column or equally in both, the apex of the right column was divided in six cats at the level of the first lumbar segment. The results are expressed in table 3. The animals were allowed to live from twelve to forty days before the vasomotor tests were made. The pressor reflexes obtained from the sciatics were less extensive than those from the brachial nerves and those from the right sciatic somewhat less extensive than those from the left. These results agree with those obtained from cats with laterally hemisected cords and show that the conduction of the afferent pressor impulses is bilateral but somewhat better on the homolateral side.

TABLE 3

Reflex changes in blood pressure after section of the apex of the right posterior gray column at the level of the first lumbar segment. Strong faradic stimulation, secondary coil at 5. Rise or fall in pressure expressed in millimeters of mercury

CAT NO.	RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVES
39	−20	+12	+20
40	+16	+18	+30
46	+ 8	+14	+16
47	+10	+20	+12
48	0	+10	+28
49	+14	+22	+28

THE PRESSOR ARC AND REFLEX

In this series of papers we have shown that the afferent impulses which cause a rise in blood pressure are initiated by strong stimuli. This is in keeping with the results of many other investigators. Martin and Lacey (11) have shown that the threshold for the pressor reflex is about 280 units as compared to 8.7 units for the depressor reflex, i.e., about thirty times higher for the former. In a few cases we have obtained a rise in pressure from very weak stimulation but in these it was evident that some unusual factor was at work. Not only was the stimulus too weak to give a true pressor reflex, but the rise was not obliterated from the sciatics by a lesion which did obliterate it in the other cats. So many factors enter into the determination of blood pressure that great care must be used in such a study as this to eliminate everything but the true vasomotor reflexes. We believe that the more successfully this is done the more evident it will become that the pressor reflex is elicited only by strong stimuli.

We have also shown that the afferent impulses, initiating the pressor reflex, are carried in the spinal nerves by the unmyelinated fibers (8). These are the fibers that mediate protopathic sensation. They enter the spinal cord along the lateral division of the dorsal root and run into the fasciculus dorso-lateralis of Lissauer in the apex of the posterior horn. In this they run for only a short distance before they end, probably in the substantia gelatinosa Rolandi. The apex of the posterior horn consists of Lissauer's fasciculus dorso-lateralis and the substantia gelatinosa and receives the afferent pressor impulses as they enter the spinal cord.

The pressor pathway up the cord is composed of these same apical structures. The tract of Lissauer consists not only of unmyelinated fibers from the dorsal roots but also of many fine endogenous fibers. These link one level of the substantia gelatinosa with another. In this way a chain of short neurones is established in the apex with the fibers in Lissauer's tract and the synapses in the substantia gelatinosa. There is reason for believing that this chain forms a path for the protopathic impulses involved in purely spinal reflexes; and it has been shown that this is the pathway up the cord for the afferent pressor impulses.

From table 1 of this paper we may conclude that these impulses ascend in the spinal cord to the thoracic segments, where a small percentage of them find their way forward through the gray matter (*g*, figure 1, page 2, of this issue) to the efferent vasomotor neurones of the white rami; but the greater volume of these impulses continues to ascend along this apical path to a vasoconstrictor center in the brain (5).

It has been said that this path consists of a series of short neurones linked together, The many synapses involved in this path offer a high resistance and are, we believe, the explanation of the high threshold for pressor stimuli. No other explanation has ever been offered for the fact that the threshold for these stimuli is thirty times greater than that for the stimuli adequate to produce the depressor reflex or the flexion reflex. In these numerous synapses we probably have the explanation of the latent period of the pressor reflex. That the latent period for the pressor reflex is greater than that for the depressor reflex, at least for stimuli of moderate intensity, has been very clearly demonstrated by Martin and Mendenhall (13). This is easily understood when it is known that the depressor path consists of long fibers and the pressor path of short fibers and many synapses.

The efferent path from the vasoconstrictor center to the efferent neurones of the white rami lies in the ventral or lateral funiculi but its exact position has not been determined. A diagram of this pressor reflex arc has been given in one of the preceding papers, figure 1 on page 2 of this issue.

We are not able to locate the vasomotor center. We know that there is located in the neighborhood of the facial colliculus a center which is responsible for the maintenance of blood pressure, the vasoconstrictor center of Dittmar (10). But when the floor of the fourth ventricle is explored with a needle electrode the only point which readily gives a rise in blood pressure is the fovea inferior. It may be that Dittmar's vasoconstrictor center is a vasotonic center and that a reflex vasoconstrictor center is located in the region of the fovea inferior. But no convincing evidence has been presented to show that this is the case.

THE DEPRESSOR ARC AND REFLEX

The depressor reflex can be produced with great regularity by weak stimulation of afferent spinal nerves. Vincent and Cameron (3) seem to regard this as a somewhat unusual reaction. They say:

> We have not always found it possible to satisfy ourselves as to the different effects of weak and strong currents respectively by means of sliding the secondary coil up to or away from the primary, but we have on several occasions succeeded in demonstrating a marked qualitative difference in effect, using in the one case a single weak cell, in another one or more strong cells.

In our own work with cats we have found that if sufficient care be taken to have all conditions favorable weak stimuli give drops with great regularity and the strength at which the maximum drop is obtained is approximately the same for all cats. This is now so well established (2) that it is not necessary to present additional evidence.

Vincent and Cameron cast doubt on the vasomotor character of the depressor reaction obtained fron spinal nerves by showing that the excessive respiration which sometimes results from stimulation of a sensory nerve can produce a drop that closely simulates the depressor reflex. They deserve great credit for calling attention to this source of error, but it would be a mistake to ascribe all drops obtained from spinal nerves to this source. They say that they have never been able to notice the drop from weak stimulation of the spinal nerves when the thorax has been opened and the effect of excessive respiration has thus been eliminated. They call attention to the fact that curare, which stops respiratory movements, also eliminates the drop from weak stimulation of the spinal nerves.

All this might be taken to indicate that a true depressor reflex can not be obtained from the spinal nerves. But a study of respiratory and blood pressure tracings of a series of cats subjected to weak stimulation of the sciatic shows conclusively that the drop to be obtained from weak stimulation of the spinal nerves is independent of the respiratory movements. When the stage of anaesthesia is most favorable for the development of the depressor reflex the weak stimuli used often do not affect the respiration at all and quite as often inhibit as increase it. The drop in blood pressure is obtained as easily when the respiration remains unaffected or is inhibited as when it is slightly increased. With stronger stimulation the greatly increased respiratory movements may no doubt play an important part in the drops in blood pressure which sometimes occur. Such respiratory effects play a part in the drops in blood pressure recorded in table 1 of this paper and table 6 of the first paper of the series (4). Wherever such strong stimuli are used that excessive respiratory movements are induced great care must taken in interpreting the results.

Weak stimulation of the vagus has the same effect as weak stimulation of the sciatic. Even greater drops in blood pressure can be obtained by moderately strong stimulation of the cat's vagus, to which very strong stimuli must be applied before a rise is produced. Only a fall in pressure can be obtained by stimulation of the depressor nerve. This difference in the afferent nerves has long been a matter of discussion and has been attributed to the existence of two kinds of afferent vasomotor fibers, with a different distribution in the peripheral nerves. We will now examine the evidence in favor of this time honored conception of special afferent pressor and depressor fibers.

a. Are there special afferent depressor fibers? Reid Hunt (1) is largely responsible for the prevalence of the conception that there are special afferent depressor fibers, since he assembled in good form the evidence available at that time. This in brief is as follows: Weak stimulation of most afferent nerves gives a drop in blood pressure. This can be obtained with stronger currents and with greater regularity if the nerves are cooled to about 5°C. At a certain stage in the regeneration of a nerve stimulation can be made to give a drop but not a rise in blood pressure. A drop is more easily obtained from the vagus than the spinal nerves and is always the result of stimulation of the depressor nerve. It was thought that these results might be most easily explained on the assumption that there were two kinds of afferent vasomotor fibers. If one made the additional assumption that the afferent pressor fibers are rendered nonconductive by cooling while the conductivity of the depressor fibers is unaffected or enhanced, and the further assumptions that the depressor fibers regenerate more rapidly than the pressor fibers, and that the depressor fibers are stimulated by weaker currents than the others, one would be in position to explain most of the facts then known. A weak stimulus applied to an afferent nerve would stimulate only the depressor fibers and give a drop in blood pressure, or if the nerve was cooled and the pressor fibers rendered non-conductive the impulses carried by the depressor fibers would cause a drop. At a certain stage in regeneration of a nerve only depressor fibers would be present and their stimulation would cause a drop. One would only need to make the one additional assumption, that these depressor fibers are alone present in the depressor nerve, and predominate in the vagus and glossopharyngeal while the pressor fibers predominate in the spinal nerves, to be in position to explain the differences in the vasomotor reactions normally obtained from these nerves.

But if we examine the facts carefully we are not likely to take so much for granted. It is difficult to believe that two afferent fibers should be so differently constituted that the one would lose its conductivity by being cooled, to 5°C. while the other would have its conductivity unimpaired or enhanced by the same temperature. So far as we know lowering the temperature decreases the conductivity of all nerve fibers. Cooling a nerve would decrease the strength of the impulses passing over it so that the net result would be the same as that of weak stimulation. The conductivity of a regenerating nerve is less than that of a normal one and only weak impulses could be made to reach the cord by way of such a nerve. It all reduces itself then to a matter of the strength and perhaps the volume of the afferent impulses which reach the spinal cord.

To our mind the most serious difficulty in the way of Hunt's explanation is to be found in the results of Martin and Lacey (11). According to them the threshold for the depressor reflex is 8.7 units as compared with 5.2 for the flexion reflex and 280 units for the pressor reflex. That is to say the depressor threshold is about the same as that of other reflexes while the pressor threshold is enormously higher. They say that stimuli 200 times above the depressor threshold are often used in experiments with the pressor reflex. It is difficult to conceive of two afferent fibers so differently constituted that the stimulation threshold of one should be 30 times greater than that of the other. Such differences in stimulation threshold may exist in end organs but not in nerve fibers.

As we have said all the facts so far as the spinal nerves are concerned are more easily explained on the basis of the strength and perhaps volume of the afferent impulses reaching the cord, and from our work on the dorsal roots we believe that it is only the protopathic afferent impulses that are here concerned. We still have to account for the segregation in the cord of afferent pressor and afferent depressor impulses and for the special reactions obtained from certain of the cranial nerves.

b. The afferent depressor path in the spinal cord. It has been shown (12) that the afferent impulses which produce the depressor reflex travel up the cord in the superficial portion of the lateral funiculus along a tract composed of long fibers with few relays. The resistance of this pathway is low as compared to that of the pressor path with its many synapses. Protopathic impulses on reaching the cord are received by the substantia gelatinosa from which two paths are open for them to the vasomotor centers: a path of low resistance in the lateral funiculus leading to the vasodilator center[1] and a path of high resistance in the apex of the posterior gray column leading to the vasoconstrictor center.

Weak impulses reaching the cord find their way along the former only and vasodilation (13) with a drop in blood pressure results. Whether they also inhibit the tonic action of the vasoconstrictor center is not so clear (14). With stimuli of moderate intensity some impulses force their way up the pressor path and produce vasoconstriction. Constriction and dilation balance each other with little change in pressure. There is reason for believing that the vasodilation is masked in this way rather than inhibited. But the failure of stimuli of a certain medium intensity to produce any change in blood pressure could be accounted for just as well by the assumption of a mutual inhibition. With strong stimuli, the resistance of the pressor path is overcome and the vasoconstrictor center brought into full action resulting in a rise in pressure. Here again it is not neccessary to assume the concomitant inhibition of the vasodilator center. The work of Martin and Mendenhall (13) would indicate that its action is only masked by the vasoconstriction, and table 1 of this paper shows that strong stimuli are very effective in producing a fall in blood pressure if the pressor path is interrupted. Vasoconstrictor fibers are much more widely distributed and their action is by its very nature more powerful than that of the vasodilators. When the vasoconstrictor apparatus is brought into full action by strong stimulation it is able to manifest itself in a rise of blood pressure irrespective of the action of the vasodilator center.

In brief we wish to explain the fact that weak stimulation of an afferent spinal nerve causes a drop while strong stimulation causes a rise in blood pressure on the basis of demonstrated spinal paths, the one with a low and the other with a high resistance. When one considers the synaptic resistance to be overcome in the pressor path, it is easy to understand why the threshold for pressor reflexes is 30 times higher than that for depressor reflexes. It is not easy to believe that one afferent nerve fiber has a stimulation threshold 30 times higher than another. It has been demonstrated that there are two such afferent spinal vasomotor paths, it has never been shown that there are two kinds of afferent vasomotor fibers.

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[1] The work of Martin and Mendenhall shows that active vasodilation occurs, and it is immaterial whether the center is anatomically distinct or is incorporated with the vasoconstrictor center. This Journal, 1915, xxxviii, 98.

c. *Depressor reflexes from certain cranial nerves.* The vagus and glossopharyngeal nerves give depressor reflexes more readily than spinal nerves. Our experience with cats shows that while weak stimulation of the vagus gives a drop, moderately strong stimulation gives a greater drop. A branch of the vagus, the depressor nerve, most easily studied in the rabbit, gives depressor reflexes exclusively. It has been supposed that the depressor nerve was composed exclusively of afferent depressor fibers and that the vagus and glossopharyngeal nerves contained relatively more of these fibers than the spinal nerves. Since the reactions from the fifth cranial nerve are the same as those from the spinal nerves, one would assume, according to this theory, that it had the same proportion of pressor and depressor fibers as they.

But there is another and better explanation for the special vasomotor reactions obtained from the vagus and glossopharyngeal nerves. These nerves are visceral nerves and their sensory fibers connect with the visceral afferent column in the medulla oblongata, that is with the fasciculus solitarius and its nucleus. The fifth cranial contains somatic afferent fibers as do the spinal nerves; and its sensory nuclei, the chief sensory nucleus and the nucleus of its spinal tract, form the upper end of the general somatic afferent column which extends through the spinal cord and medulla into the pons. The spinal tract of the fifth is the direct upward continuation of the tract of Lissauer, and its nucleus the direct upward continuation of the substantia gelatinosa Rolandi. With this somatic afferent column the vagus and glossopharyngeal nerves have only the very slightest connections. We have seen that the pressor pathway is intimately associated with this somatic afferent column and it is the somatic afferent nerves that most readily give pressor reflexes. The visceral nerves having only very slight connections with the somatic afferent column, give pressor reflexes with more difficulty.

As has been pointed out by Martin and Mendenhall (13) the high blood pressure produced by general vasoconstriction is designed to supply the somatic musculature with sufficient blood during a period of muscular effort. We will quote this suggestive paragraph:

> It may not be amiss to point out that an important feature of the physiological significance of high blood pressure induced by vasoconstriction is in the diversion of much of the blood of the body into those organs which appear to have a vasodilator innervation but no constrictor: namely, the skeletal muscles.

Impulses from visceral nerves do not lead directly to activity of the skeletal muscles. It is the somatic afferent nerves which carry impulses initiating skeletal reflexes. Painful stimulation of the surface of the body initiates the movements necessary for escape or combat and at the same time brings about vasoconstriction and high blood pressure that the skeletal muscles may be better supplied with blood during the emergency. Reasoning in this way it is easy to understand why it is only the somatic nerves that have direct connections with the vasoconstrictor center. We believe that the known differences in the central connections of the visceral afferent and somatic afferent nerves is sufficient to account for the difference in the vasomotor reactions obtained from them without assuming that the one contains a larger number of special afferent depressor fibers than the other.

d. The vasodilator center. Weak stimulation of afferent nerves produces an active vasodilation (13). This implies the existence of a center for vasodilator reflexes. It is not known whether this center is anatomically distinct or incorporated within a general vasomotor center, Bayliss (14), Fananow and Tschalusson (14), and Martin and Stiles (15). We have shown that there exists in the floor of the fourth ventricle within the area postrema just lateral to the obex a "depressor point" direct faradic stimulation of which will cause a drop in blood pressure (9). Three millimeters higher up at the fovea inferior is the "pressor point" direct stimulation of which causes a rise in blood pressure. While the reactions to be obtained from these two points are highly specific we are not yet prepared to assert that they represent the location of the vasodilator and vasoconstrictor centers, although it is not improbable that such is the case.

e. The vasomotor balance. Many facts point to the conclusion that with weak stimulation the depressor apparatus alone is brought into play, while stronger stimulation gives a change in blood pressure which represents the balance between the action of the pressor and depressor mechanism. Martin and Mendenhall (13) are also of the opinion that pressor and depressor influences can be aroused simultaneously and "the resultant effect on the peripheral vasomotor mechanism depends on the balance that is established between the opposing influences." Their results indicate that with the strong stimuli the action of the vasodilator center is masked rather than inhibited.

Our experiments have shown that when the depressor path in the cord has been divided in the upper lumbar region, stimuli of moderate strength will give a greater rise in blood pressure from the sciatic than from the brachial nerves (12). These results are most easily understood by assuming that with stimuli of moderate strength the change in blood pressure represents a balance between the action of the pressor and depressor impulses and that when the depressor impulses have been cut out the pressor impulses manifest themselves in a greater rise in blood pressure.

A glance at table 1 of this paper will show that when the pressor path has been interrupted in the lower thoracic or upper lumbar segments of the cord strong stimulation which gives a good pressor reflex from the brachial nerves will usually give a very considerable drop from the sciatic nerves. This would indicate that strong stimuli are at least as effective in exciting the depressor apparatus to activity as weak stimuli, but when the pressor mechanism is intact the net result is a rise.

In line with this reasoning is the fact that when one stimulates a spinal nerve with weak faradic currents an optimum strength can be found which will give the greatest drop. With increasing strengths the drop decreases in extent and finally gives place to a negative reaction, to a slight reaction which is variable in direction, or to a drop followed by a rise. With still stronger stimuli the pressor reflex appears and increases with the stimulus until a certain optimum strength is again reached. The point at which the reaction is negative or variable represents the strength at which the activity of the pressor balances that of the depressor apparatus.

THE AFFERENT PATH TO THE RESPIRATORY CENTER

We have not been able to locate any special part of the cord through which impulses ascend to the respiratory center. Bilateral section of the apices of the posterior horns interferes somewhat with the conduction of these impulses. In the same way and to about the same extent bilateral lesions in the lateral funiculi impede their conduction. These results indicate that the fibers which carry these impulses are widely scattered through the spinal cord and do not form a single compact fascicle.

THE CONDUCTION OF PAIN IN THE SPINAL CORD

The conditions under which painful afferent impulses are propagated along the spinal cord are not the same in the carnivora as in man. It is well established that in man pain impulses are carried by the lateral spino-thalamic tract (16). This is composed of long fibers which, beginning in the various segments of the cord, cross the median plane and extend uninterruptedly to the thalamus in the ventral part of the lateral funiculus. A lesion in this part of the cord in man produces total analgesia of the opposite side of the body below the lesion. In the cat, on the other hand, hemisection does not produce an appreciable degree of hypalgesia in either hind leg.

The conduction of pain in the cat's cord is bilateral and effected through a series of relays which give an opportunity for the impulses to enter the gray matter and cross the cord from side to side. This is clearly shown by the work of Karplus and Kreidl (17) who hemisected the spinal cord on the left side in the upper cervical and on the right side in the lower thoracic region. Cats, in which both sides of the cord had been thus divided and in which all long conduction paths to the brain had been cut, were still able to feel pain in the hind limbs. They conclude that pain is conducted in the cat's cord by a series of short fibers of the funiculus proprius system with frequent relays through the gray substance.

A study of the sensibility of the hind limbs in cats with various cord lesions confirms these conclusions. We could observe no hypalgesia after posterior hemisection (4), after lateral hemisection (4) or bilateral section of the lateral funiculi (12). We have repeated the double hemisection of Karplus and Kreidl on three cats and can confirm their results.

It would seem that the paths for pain conduction in the cat's cord are more diffuse than those for the conduction of the afferent vasomotor impulses. The latter are readily eliminated by appropriate lesions while nothing short of complete transection will stop the conduction of pain.

The most interesting observation in regard to the perception of pain was that after section of both posterior gray columns in the thoracic or upper lumbar regions the hind limbs were hyperaesthetic for a few days. The hyperaesthesia was noticeable shortly after the animal came out from under the anaesthetic and was usually at its height twenty-four hours after the operation. It gradually decreased and could not be detected after a week or ten days. It is well known that an injury producing a hemisection of the spinal cord in man results in a more or less transient hyperaesthesia on the paralyzed side from the lesion down (18). It is probable that the hyperaesthesia observed by us in the cat is of the same sort and due to the same cause.

No satisfactory explanation has yet been given for the phenomenon in man. In our cats the hyperaesthesia was caused by the lesions in the posterior horns. We believe that all protopathic impulses are received in the cord through the tract of Lissauer and the substantia gelatinosa Rolandi (8), and that part of these impulses ascend in this apical complex to produce spinal and bulbar reflexes while part of them are transferred to special fiber tracts such as the pain path and the depressor pathway. It seems probable that when the pathway in the apex of the posterior horn is blocked a larger proportion of these impulses finds its way into the path for conscious pain. On the other hand it may be that the lesion increases the irritability of this apical system, through which the protopathic impulses pass on entering the cord. In favor of this latter explanation is the fact that the hyperaesthesia disappears in ten days or two weeks. Whatever the explanation, the fact remains that section of the apices of the posterior horns produces a transient hyperaesthesia of the body below the lesion. This fact is in line with our contention that the primary spinal mechanism for the reception of protopathic impulses is formed by the tract of Lissauer and the substantia gelatinosa which form the apex of the posterior horn.

A phenomenon, which is probably only another expression of this hyperaesthesia, has been mentioned in the fourth paper of this series (5). Figure 3 of that paper shows that, while stimulation of the brachial nerves gave a smooth pressor curve, stimulation of the sciatic gave an irregular curve the sharp rises in which were due to muscle spasm. In these cats with lesions in the apices of the posterior horn muscle spasms are much more likely to result from the stimulation of the sciatic than the brachial nerves. The muscle spasms are more marked if the experiment is made within the first few days after the operation at a time when the hyperaesthesia is evident, and we believe that the two phenomena have the same basis.

CONCLUSIONS

1. Afferent pressor impulses travel up the spinal cord in the apex of the posterior horn on both sides of the cord but somewhat better homolaterally.

2. It is probable that a small portion of the pressor impulses passes directly forward through the gray matter to the efferent vasomotor neurones of the white rami.

3. Most of the pressor impulses ascend through the apical path in the posterior horn to the vasoconstrictor center of the brain.

4. There is some evidence which points to the location of the reflex vasoconstrictor center in the region of the foeva inferior in the floor of the fourth ventricle.

5. The efferent path from the vasoconstrictor center to the vasomotor neurones of the white rami is located in the ventral or lateral funiculi, certainly not in the posterior.

6. The depressor path is located in the ventral part of the lateral funiculus and its conduction is bilateral although somewhat better on the contralateral side as indicated by the results of hemisection.

7. There is some evidence that a separate vasodilator center may exist in the region of the area postrema lateral to the obex in the floor of the fourth ventricle.

8. Weak stimulation of an afferent spinal nerve causes a drop in blood pressure which is a true vasomotor reflex and independent of respiratory movements.

9. A drop in blood pressure obtained from strong stimulation may be due to excessive respiratory movements.

10. True pressor reflexes are produced only by strong stimulation.

11. There is little evidence in favor of the time honored conception of two kinds of afferent vasomotor fibers and considerable evidence against it.

12. We have presented a theory of the vasomotor mechanism which accounts for the qualitative difference in the results of weak and strong stimuli on the basis of the resistance offered by the depressor and pressor paths respectively.

13. The glossopharyngeal and vagus nerves and the depressor branch of the latter are all visceral nerves and are connected with the visceral afferent column in the medulla while the fifth cranial and the spinal nerves are all connected with the somatic afferent column. This difference in the central connections of the two groups of nerves

is sufficient to account for the difference in the vasomotor reactions obtained from them.

14. No clearly defined path for afferent impulses to the respiratory center could be found. The fibers which carry these impulses seem to be somewhat widely scattered in the cord.

15. In the cat pain conduction in the spinal cord is bilateral and effected through a series of short relays.

16. For a few days following bilateral injury to the apices of the posterior gray columns there is hyperaesthesia of the hind limbs and all parts of the body behind the lesion.

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OSCILLATORY VARIATIONS IN THE CONTRACTIONS OF RHYTHMICALLY STIMULATED MUSCLE

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In 1904, Storey (1) reported that when electrodes are thrust into the gastrocnemius muscle of the cat and the muscle is stimulated by currents derived from a magneto-machine, the successive contractions rise and fall in "tonus rhythms." These rhythms, in Storey's experiments, began immediately after stimulation began, before fatigue occurred, and continued although the sciatic nerve was severed. A somewhat similar phenomenon was described by Symons (2), who noted that when certain frog, toad and mammalian muscles are fatigued with maximal break induction shocks temporary wave-like variations in the summit line can be produced in the later stages of fatigue by altering the load, the rate of stimulation, the temperature, or by introducing short periods of rest or of submaximal stimulation. Conditions inducing more rapid fatigue hasten the stage when the waves appear. Symons' observations on frog's muscle were confirmed by Burridge (3), who found the waves especially marked in fatigue curves obtained by perfusing the muscle with acid phosphates, carbon dioxide in Ringer's solution, and with lactic acid. Since the waves were not produced by perfusion with neutral salts (potassium chloride, sodium lactate, etc.) and since all the substances except ammonium lactate which produced the waves contain the hydrogen ion, the inferences were drawn that the hydrogen ion is a necessary concomitant of the waves, and that when they appear free acid is in process of neutralization by ammonia.

During recent experimentation on the effects of adrenalin on muscular fatigue we have subjected the tibialis anticus muscle, either by direct stimulation or by stimulation through its nerve, to rapidly and rhythmically repeated single stimuli, varying from about 35 to about 300 per minute. Both cats and dogs were used in the experiments, the former much more than the latter. The animals were anesthetized

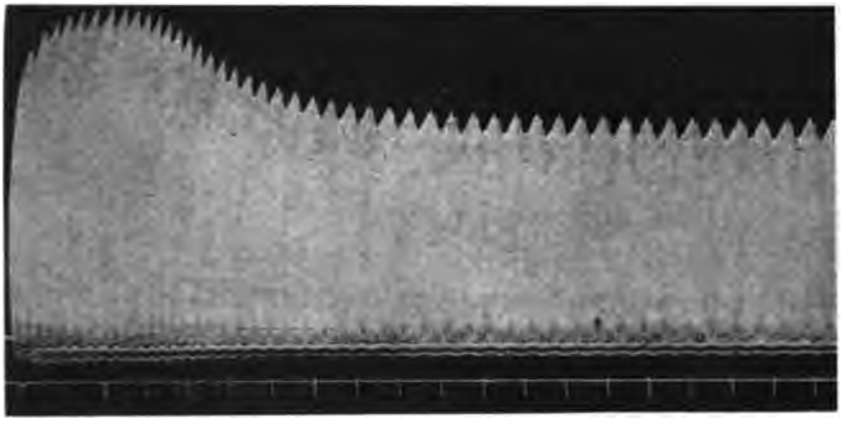

Fig. 1. Appearance of waves at beginning of contraction; waves longer as contraction continues. Record of tibialis anticus muscle stimulated 150 times per minute, and extending a spring having an initial tension of 120 grams. This record was taken from the beginning of the experiment and shows the "treppe" or initial improvement of the muscle. The waves persisted for about one hour and fifty-three minutes of continuous stimulation. Here and in other figures time is recorded in half-minute intervals.

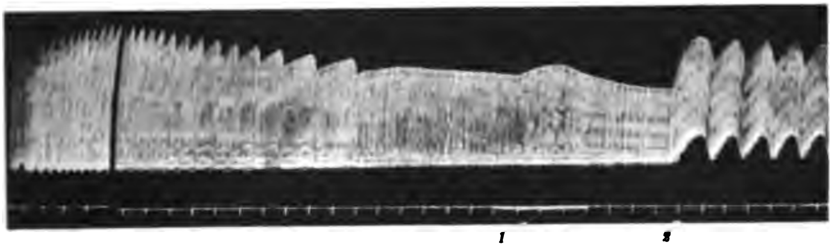

Fig. 2. Reappearance of waves when pull on muscle slightly increased. Record of tibialis anticus muscle, contracting 120 times a minute, and extending a spring having (as an after load) an initial tension of 100 grams. At *1* splanchnic stimulation began and continued for two minutes. At *2* the tension of the spring changed to pull continuously on the muscle (i.e., the muscle was "loaded").

with urethane (2 grams per kilo by stomach), or were studied without an anesthetic after being decerebrated under ether. No difference was noted between results obtained in these conditions. Only the tendon was exposed, through a slit in the skin; the contractile portion of the muscle was left *in situ*, supplied normally with its natural blood.[1] While at rest the muscle was slightly stretched, but otherwise not in stress; as soon as it began to contract it pulled against a spring having usually an initial tension of 100 grams. A characteristic feature of the muscular response under these circumstances was an oscillatory variation in the height of contraction (4). At present we are not able to offer any explanation of this phenomenon, but since we have noted a number of conditions which affect its appearance, disappearance, and alteration, an account of the phenomenon and some circumstances which influence it is now presented.

APPEARANCE AND DISAPPEARANCE OF WAVES

Conditions for the appearance of the oscillations. In our experience the oscillations have appeared most prominently in strong vigorous animals, and they have failed to appear only in a few animals that were sickly or asthenic at the time of observation. They begin to be manifested during the first contractions of the muscle while the initial improvement in the degree of shortening is still in progress, and therefore before fatigue, in the sense of lessened efficiency, has begun (see figs. 1 and 2).

As Cannon and Nice have already pointed out, if a fatigued muscle is shortening in a uniform manner, so that no oscillations are present, injection of a small dose of adrenalin or stimulation of a splanchnic nerve may promptly result in the development of rhythm (5). The renewal occurs although the arterial pressure in the muscle is kept at an even level.

Increase of tension may start waves again after they have ceased. In the instance represented in figure 2, the muscle at first was contracting 120 times a minute and pulling against a spring having (as an afterload) an initial tension of 100 grams. The waves ceased after about seven minutes. In the following six minutes, the height of contraction was increased as a result of splanchnic stimulation, but the oscillations did not come back. Then the check for the spring was removed and its tension thus continuously applied to the muscle, and immediately the oscillations reappeared. There is apparently a limit to the amount of tension which can be applied to the muscle, favorable to the development of oscillations, for we have also noted that when they were present in an afterloaded muscle, they disappeared as soon as the muscle was loaded (see fig. 3).

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[1] For a detailed description of the method employed, see Cannon and Nice: This Journal, 1913, xxxii, 45; and Gruber: ibid., 221 and 438; ibid., 1914, xxxiii, 336; ibid., 1914, xxxiv, 89.

Conditions for the disappearance of the oscillations. After the oscillations have begun they usually continue for a considerable period after the fairly even height of contraction due to fatigue has been reached (see fig. 1). The period during which they continue thus before being gradually obliterated varies in different animals. In the animal from which figure 1 was taken the waves were present during continuous stimulation for one hour and fifty-three minutes. In other animals the waves have been present as long as five hours. The impression which numerous observations have made upon us is that the persistence depends directly on the vigor of the animal. In several animals weakened by fasting we have seen the waves begin as usual with the first stimulations and quickly vanish before the fatigue level had been reached. In one cat, without food six days, nine oscillations were recorded, whereupon the muscle began to contract to a smooth and even line; and in another animal, without food four days, only two oscillations occurred, lasting one minute (see fig. 4).

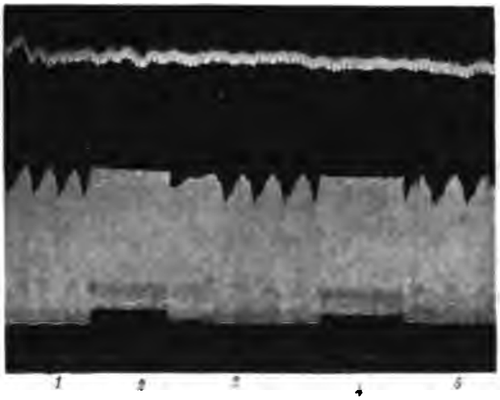

Fig. 3. Disappearance of waves with increase of pull on muscle. Upper record, blood pressure with mercury manometer. Lower record, the tibialis anticus muscle contracting 120 times a minute and extending a spring with an initial tension of 120 grams. At *1*, *3* and *5* the muscle was after-loaded, at *2* and *4* the waves disappeared as the muscle was directly loaded.

A strong dose of adrenalin (1 cc., 1: 10,000, intravenously), sufficient to cause a marked constriction of the arterioles, results in lessened height of contraction and slowing of the waves, and, if this decreased

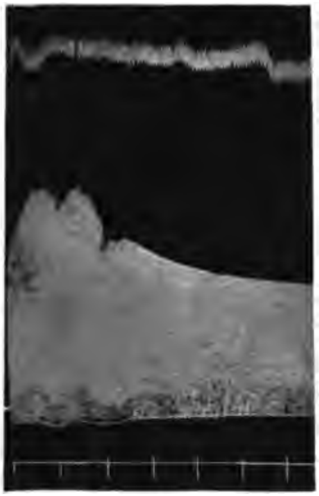

Fig. 4. Influence of general condition on muscle waves. Upper record, blood pressure. Lower record, contraction of the tibialis anticus muscle (228 times a minute, extending a spring with an initial tension of 120 grams) in an animal fasted four days.

blood supply persists for a long time, in a banishment of the oscillations; but within a few minutes they start again (see fig. 5). This observation indicates that a sufficient supply of blood is a necessary condition for the existence of the waves, for in this case, although muscular contractions continued in spite of defective blood supply, the oscillatory variations in contractions did not. Nevertheless we have seen the waves in cases of low arterial pressure after the brain and upper spinal cord had been pithed, and also in excised perfused muscles.

The suggested relation of the oscillations to the arterial blood supply might indicate that the ups and downs of arterial pressure, especially after injection of adrenalin, would account for the variations in height of the muscle record (6). We have observed the rhythmic variations in muscular contraction, however, without any corresponding changes in the arterial pressure curve (see figs. 3, 4 and 6), and also we have records of rhythmic alterations in arterial pressure without corresponding waves in muscular activity (see fig. 5).

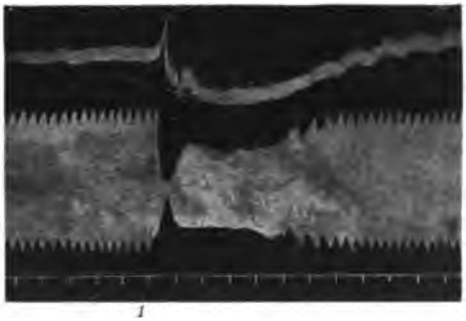

Fig. 5. Influence of diminished blood supply on muscle waves. Upper record, blood pressure. Lower record, the tibialis anticus muscle contracting 300 times a minute. At *1* adrenalin (1 cc., 1: 10,000, injected intravenously) abolished the waves but not the contractions. The waves gradually returned to their former rate.

Multiple oscillations. The records obtained from muscles, to which stimuli of uniform strength were applied at a uniform rate, were not always simple oscillations as observed in figure 1. Double and modified waves are frequently observed (see figs. 6 and 8). In figure 6 seven small waves are superimposed upon each large wave, which lasted about one minute. Some muscles recorded a

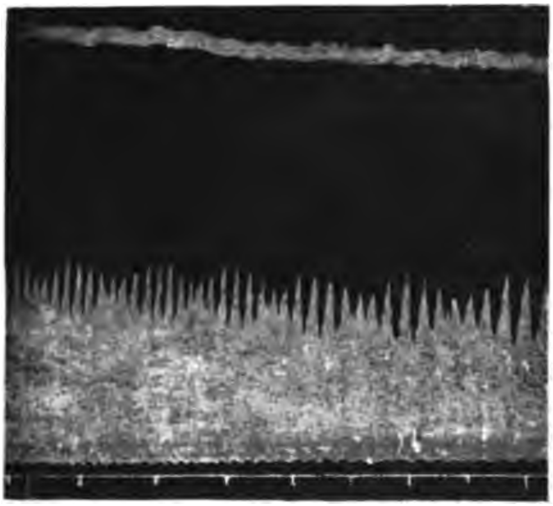

Fig. 6. Compound muscle waves. Upper record, blood pressure. Lower record, the tibialis anticus muscle contracting 240 times a minute, and extending a spring with an initial tension of 120 grams.

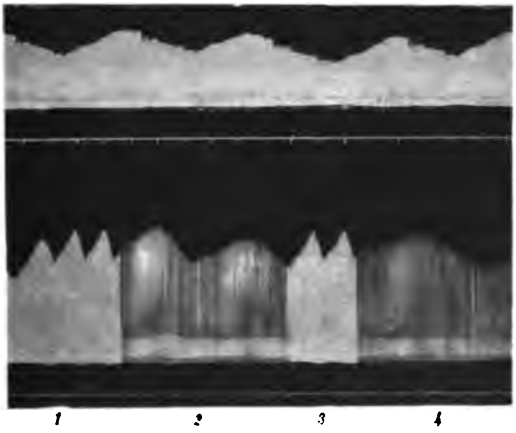

Fig. 7. Influence of rate of stimulation and of tension on duration of muscle waves. Lower record, muscle contracting, at *1* and *3*, 130 times a minute, and extending a spring with an initial tension of 100 grams. At *2* and *4* the stimulation rate was lowered to 40 per minute.

Upper record, same conditions as at *1* and *3* in lower record, except that initial tension of spring is 180 grams.

rhythmic alternation of a high and a low wave; and others contracted rhythmically in a "treppe" like manner in three or four wave groups each followed by a deep notch. Splanchnic stimulation, with the adrenal gland intact, sometimes changed these modified rhythms into the usual even and regular rhythm.

CONDITIONS AFFECTING THE OSCILLATORY RATE

Conditions causing an increase of rate. The waves are always faster at the beginning of the stimulation, when the muscular contractions are high and the initial improvement is in progress. At this point of maximal efficiency of the muscle, they occur at the rate of 2 to 20 per minute; they gradually decrease in rate as fatigue approaches and the height of the muscular contraction becomes low, until they finally disappear.

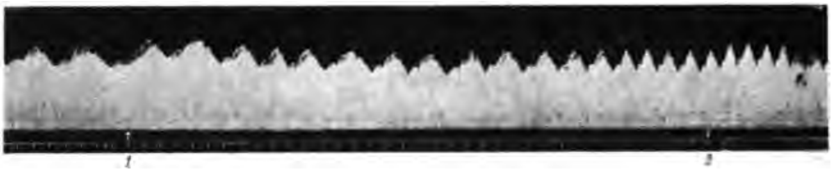

Fig. 8. Influence of small doses of adrenalin on rate and height of muscle waves. Record of the tibialis anticus muscle contracting 120 times a minute and extending a spring with an initial tension of 120 grams. At *1* and again at *2* adrenalin (0.3 cc., 1: 100,000) was injected intravenously.

Symons (2) observed that if the rate of stimulation is increased the wave-time decreases, i.e., the waves recur more rapidly. These observations are corroborated by our experiments. If the muscle is not too fatigued and the rate of stimulation is increased the waves appear at a much more rapid rate. This increase in rate has an optimal point beyond which the muscle rapidly fatigues to a smooth even curve. The effect of changing the rate of stimulation upon the waves can be seen by reference to figure 7. The initial tension was 100 grams. When the rate of stimulation was 130 per minute (at *1* and *3*), one wave lasted for about thirty seconds; but when the rate was reduced to 40 per minute (at *2*), a single wave lasted about ninety seconds, i.e., when the rate of stimulation was decreased from 130 to 40 per minute the duration of a single wave was correspondingly increased, lasting three times as long as before.

The tension against which the muscle contracts alters the rate of the waves. With a constant rate of stimulation a decrease of tension increases the rate of the waves and an increase of tension decreases the rate. In figure 7 the rate of stimulation is 130 per minute in regions *1* and *3* in the lower curve and throughout the upper curve. The tension at the designated regions in the lower curve is about 100 grams and there result about two waves to the minute. In the upper curve the tension is 180 grams and one wave lasts about two and a half minutes.

Our results do not substantiate Symons' inference (7) that adrenalin has no effect upon the wave-like variations. We have observed that an injection of a small dose of adrenalin (0.1 to 1.5 cc., 1: 100,000, intravenously) or splanchnic stimulation or increased blood pressure may not only reëstablish the waves after they have disappeared (see p. 38), but increase the rate of the oscillatory rhythm if already present (8). In figure 8 two injections of adrenalin (0.3 cc., 1: 100,000, intravenously) were made. After the first injection (at *1*) there resulted a gradual increase in the wave rate. These waves, however, did not reach their maximal rate until after the second injection of adrenalin (at *2*). Upon further injection of adrenalin no additional increase was observed.

In some muscles when the tension was changed from an after load to a direct load the waves increased in rate and *vice versa*. This however is not always the case (see fig. 3).

Conditions causing a decrease of rate. The most prominent factor in bringing about a slowing in the wave rate is the oncome of fatigue. Under uniform stimulation the waves gradually increase in length, until the waves may last four or five minutes each and then finally disappear. Although Symons attributed these waves to fatigue, he noticed that fatigue also increases their duration. If the rate of stimulation is made slower, or the tension is increased with a uniform and constant rate of stimulation, the waves are prolonged (see fig. 7). Long waves were observed at the start in an animal that had been fasting (see fig. 4), and in normal animals after the blood supply had temporarily been shut off, and after a large dose of adrenalin had been injected intravenously (see fig. 5). In many animals these two latter conditions brought about complete obliteration of the waves.

OSCILLATIONS IN ISOLATED, DENERVATED AND CURARIZED MUSCLES

Not only were the waves observed in normal muscles (*in situ*), but also in muscles excised and perfused with Ringer's solution at 38.5°C. and at a pressure of 95 mm. of mercury (see fig. 9). This substantiates the earlier statement that the waves are not dependent upon rhythmic alterations in arterial pressure but are the result of some property or properties of the muscle tissue itself. Since the waves are seen in denervated muscles either after nerve degeneration (9) (nerves cut nine to fifteen days before the experiment), or after paralysis of the nerve endings by curare it is evident that changes in irritability or conductivity of the nerve-trunks or nerve-endings supplying the muscle do not govern their appearance.

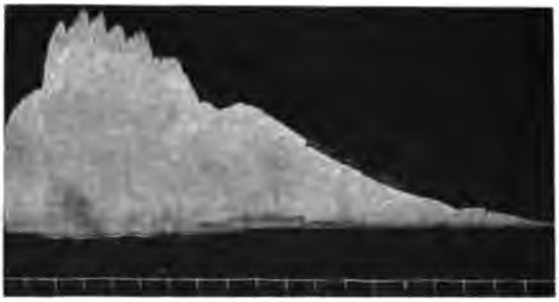

Fig. 9. Record of excised, perfused muscle contracting 120 times a minute and extending a spring with an initial tension of 120 grams.

SUMMARY

Wave-like variations in the height of contraction are obtained from mammalian muscles under rhythmical and uniform stimulation. These waves are similar to the waves observed by Symons and Burridge in their experiments upon frogs. In our experiments they occur too soon to be the result of a fatigue process.

The waves are related to the condition of the animal. In healthy vigorous animals they may be present for more than four hours; in sickly or fasting animals usually no waves or only a few occur.

Increasing the initial tension against which the muscle is contracting may occasion the appearance or the disappearance of the waves.

Injection of a small amount of adrenin or splanchnic stimulation may bring about a return of the waves after they have disappeared.

Fatigue or diminished blood supply (e.g., a dose of adrenin strong enough to constrict arterioles, or low arterial pressure) bring about a slowing or an entire disappearance of the waves. In healthy vigorous animals the waves usually return in a short time after the injection of a strong dose of adrenin.

Splanchnic stimulation, injections of a weak dose of adrenin (0.1 to 1.5 cc., 1: 100,000, intravenously), or a decrease in tension, each may increase the rate of the oscillations.

The muscle waves are observed in curarized or isolated or denervated muscles; in the body they do not coincide with waves of arterial pressure. They are, therefore, strictly of muscular origin.

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FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

IX. The Effect of Adrenin on the Factors of Coagulation

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It was noted in 1903 (1) that adrenin shortens the coagulation time of blood. The careful studies of Cannon and Gray (2) have established this action, have revealed in part its mechanism, and have explained the reason for previous discordant results. They have shown that minimal doses of adrenin constantly decrease coagulation time and that apparently the liver (and intestines?) is an essential to this action of the drug. They found that in large doses adrenin increases coagulation time; and Howell (3) has described a condition akin to hemophilia which he was able to produce in dogs by the injection of massive doses. From these observations it seemed highly probable that a study of the effect of adrenin on the factors of coagulation would throw some light on the source of these factors in the animal body.

The experimental procedure was as follows: The cats were etherized and a tracheal cannula inserted. Under deep ether anaesthesia they were pithed to the midthorax through the orbit. Artificial respiration was immediately begun. The right femoral artery was then bared and a vaselined cannula inserted from which the specimens for analysis were drawn. The left femoral vein was also fitted with a cannula into which the injections of adrenin[1] were made. After about forty minutes had elapsed (to allow the animal to recover from the immediate "shock" of pithing) the coagulation time was determined. The method employed was that of drawing about 1 cc. of blood into a small test tube and inverting the tube cautiously every fifteen or thirty seconds. As soon as this determination was made the first specimen of blood for analysis was drawn. All such specimens were drawn directly into vaselined centrifuge tubes containing a 0.1 per cent solution of sodium oxalate in Ringer's solution. There was enough oxalate in the tube to make a solution of 0.1 per cent of oxalate in the blood. The tubes and cannulae were vaselined by immersing them in a saturated solution of petrolatum in ether, allowing them to drain and the ether to evaporate. Sometimes the specimens were centrifuged as soon as they were drawn and sometimes allowed to stand until the end of the experiment.

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[1] The adrenin used was that prepared and sold by Parke, Davis & Co., under the name of "Adrenalin Chloride."

After the plasma was separated by means of centrifugalization at high speed for twenty minutes, prothrombin time and antithrombin were determined by the methods of Howell (3). This procedure is in brief as follows: To 5 drops of oxalated plasma in small test tubes are added respectively 2, 3, 4, 5 and 6 drops of a 0.5 per cent $CaCl_2$ solution. The tubes are inverted every fifteen seconds and the time at which a firm clot appears is called the "prothrombin time." The first tube to clot is the one containing the optimum amount of calcium chloride and it is this time which is used as the index of the prothrombin present in the specimen.

The plasma remaining from the prothrombin determination is heated to 60°C., precipitating the proteins, is then centrifuged, and the clear supernatant fluid drawn off and used as an antithrombin solution. Varying amounts of a solution of thrombin, prepared from beef fibrin (4), were placed in small test tubes and one drop of the antithrombin solution added. This mixture was allowed to stand for fifteen minutes and then 10 drops of a solution of "dried oxalated plasma" (4) added. Five tubes were usually used. The time necessary to produce a firm clot in each tube was noted. Either the average of all five times or the average of the three middle tubes was used as an index of the amount of antithrombin present in the specimen. This determination was controlled by setting up a similar series of tubes without antithrombin. Such tubes invariably clotted in from five to ten minutes.

The fibrinogen determinations were made by the heat coagulation method of Whipple and Hurwitz (5) an unsatisfactory and unreliable method for this work, because, chiefly, the amounts of blood required for accurate results are too large to obtain from cats without introducing the factor of hemorrhage.

The "prothrombin time" (6), while in itself merely an indication of the coagulation time, can here be used as an index of the amount of prothrombin present because antithrombin in these experiments did not vary. In the first experiments the coagulation time and prothrombin time varied so much, due probably to the excitement incident to confining and etherizing the animal, that it was decided, in order to eliminate such disturbance, to cut the splanchnic nerves before beginning the experiment. Under aseptic precautions an incision was made in the anterior abdominal wall and the nerves cut where they cross the crura of the diaphragm. Both the major and minor splanchnics were severed when both were found. The latter was not found at operation in about half the animals; neither nerve was ever found intact at necropsy. The operated animals were never used for experiments until the abdominal wound was completely healed—an interval of from two to eight weeks.

The following illustrative protocols will show the course of procedure and the details of the results:

Experiment VIII. July 2, 1915. Cat 15. Weight 3 k. Both splanchnic nerves cut June 15, 1915.

9.50 a.m. Etherized.
9.58 a.m. Pithed.
10.37 a.m. C. T.—10′—10′30″.
10.47 a.m. Specimen no. 1—4.5 cc. from right femoral artery.
10.49 a.m. C. T.—7′.
10.55 a.m. 0.4 cc. *adrenin* (1: 100,000) into left femoral vein.
10.59 a.m. C. T.—3′30″—4′15″.
11.04 a.m. Specimen no. 2—4.5 cc.
11.06 a.m. C. T.—2′15″.
11.25 a.m. C. T.—3′.
11.29 a.m. C. T.—4′45″—5′30″.
11.36 a.m. Specimen no. 3—4.5 cc.
12.17 p.m. C. T.—7′—7′30″.
12.26 p.m. Specimen no. 4—4.5 cc.
12.27 p.m. C. T.—3′30″.
12.31 p.m. C. T.—5′30″—6′30″.

Autopsy shows both splanchnic nerves divided.

Prothrombin.

									Optimum	*Check*
5 gtts.	plasma	no. 1	+2	gtts.	CaCl$_2$	Clotting	time	4′		
"	"	"	3	"	"	"	"	5′		
"	"	"	4	"	"	"	"	5′		
"	"	"	5	"	"	"	"	5′30″		
"	"	"	6	"	"	"	"	5′30″	4′	4′45″
"	"	no. 2	2	"	"	"	"	3′30″		
"	"	"	3	"	"	"	"	3′		
"	"	"	4	"	"	"	"	3′		
"	"	"	5	"	"	"	"	3′		
"	"	"	6	"	"	"	"	2′30″	2′30″	2′50″
"	"	no. 3	2	"	"	"	"	3′		

									Optimum	*Check*
5 gtts.	plasma	no. 3	2	gtts.	CaCl$_2$	Clotting	time	3'30"		
"	"	"	4	"	"	"	"	2'30"		
"	"	"	5	"	"	"	"	3'30"		
"	"	"	6	"	"	"	"	3'	2'30"	3'15"
"	"	no. 4	2	"	"	"	"	3'		
"	"	"	3	"	"	"	"	3'		
"	"	"	4	"	"	"	"	2'30"		
"	"	"	5	"	"	"	"	2'30"		
"	"	"	6	"	"	"	"	3'	2'30"	3'30"

Antithrombin

									Average
10 gtts.	fibrinogen	+3	gtts.	thrombin	+1	gtt.	no. 1	30'	
"	"	4	"	"	1	"	"	30'	
"	"	5	"	"	1	"	"	20'	
"	"	6	"	"	1	"	"	10'	
"	"	7	"	"	1	"	"	10'	20'
"	"	3	"	"	1	"	no. 2	40'	
"	"	4	"	"	1	"	"	30'	
"	"	5	"	"	1	"	"	30'	
"	"	6	"	"	1	"	"	30'	
"	"	7	"	"	1	"	"	10'	28'
"	"	3	"	"	1	"	no. 3	30'	
"	"	4	"	"	1	"	"	30'	
"	"	5	"	"	1	"	"	20'	
"	"	6	"	"	1	"	"	20'	
"	"	7	"	"	1	"	"	10'	22'
"	"	3	"	"	1	"	no. 4	30'	
"	"	4	"	"	1	"	"	30'	
"	"	5	"	"	1	"	"	30'	
"	"	6	"	"	1	"	"	20'	
"	"	7	"	"	1	"	"	20'	26'

Experiment XII—Control. July 22, 1915. Cat 23. Weight 3.8 k. Both splanchnic nerves cut June 23, 1915.

10.10 a.m. Etherized.
10.15 a.m. Pithed.
10.39 a.m. C. T.—10'.
10.58 a.m. C. T.—9'—9'—12'.
11.00 a.m. Specimen no. 1–5 cc.—from right femoral artery.
11.31 a.m. C. T.—9'—9'30".
11.33 a.m. Specimen no. 2–4.5 cc.
12.08 p.m. C. T.—11'—11'.
12.10 p.m. Specimen no. 3–4.5 cc.
12.24 p.m. 0.8 cc. 0.9 per cent salt solution into left femoral vein.
12.32 p.m. Specimen no. 4. 4–4.5 cc.
12.34 p.m. C. T.—5'30"—10'—10'30".

Autopsy shows both nerves divided.

Prothrombin

									Optimum	*Check*
5 gtts.	plasma	no. 1	+2	gtts.	$CaCl_2$	Clotting	time	9'30"		
"	"	"	3	"	"	"	"	11'30"		
"	"	"	4	"	"	"	"	9'		
"	"	"	5	"	"	"	"	8'		
"	"	"	6	"	"	"	"	8'30"	8'	8'
"	"	no. 2	2	"	"	"	"	7'		
"	"	"	3	"	"	"	"	6'30"		
"	"	"	4	"	"	"	"	6'		
"	"	"	5	"	"	"	"	5'30"		
"	"	"	6	"	"	"	"	4'30"	4'30"	6'30'
"	"	no. 3	2	"	"	"	"	6'30"		
"	"	"	3	"	"	"	"	11'30"		
"	"	"	4	"	"	"	"	5'30"		
"	"	"	5	"	"	"	"	5'		
"	"	"	6	"	"	"	"	5'30"	5'	7'45"
"	"	no. 4	2	"	"	"	"	7'30"		
"	"	"	3	"	"	"	"	6'30"		
"	"	"	4	"	"	"	"	5'30"		
"	"	"	5	"	"	"	"	6'		
"	"	"	6	"	"	"	"	6'	5'30"	7'45'

Antithrombin

							Average
1 gtt.	antithrombin	no. 1	2	gtts.	thrombin	2'30"	
"	"	"	3	"	"	18'	
"	"	"	4	"	"	6'	
"	"	"	4	"	"	6'	
"	"	"	5	"	"	6'	
"	"	"	6	"	"	6'	7'30"
"	"	no. 2	2	"	"	2'30"	
"	"	"	3	"	"	6'	
"	"	"	4	"	"	6'	
"	"	"	5	"	"	2'30"	
"	"	"	6	"	"	6'	5'
"	"	no. 3	2	"	"	2'30"	
"	"	"	3	"	"	2'30"	
"	"	"	4	"	"	2'30"	
"	"	"	5	"	"	2'30"	
"	"	"	6	"	"	2'30"	2'30"

Experiment XIV. July 27, 1915. Cat 20. Weight 3 k. Both splanchnics cut June 19, 1915.

10.15 a.m. Etherized.
10.35 a.m. Pithed.
10.59 a.m. C. T—6'30"—7'45".
11.01 a.m. Specimen no. 1—4.5 cc. from right femoral artery.

11.07 a.m. 1 cc. 0.9 per cent salt solution into left femoral vein.
11.10 a.m. C. T.—5'—5'.
11.16 a.m. Specimen no. 2—4.5 cc.
11.17 a.m. C. T.—3'30"—6'.
11.49 a.m. 0.4 cc. *adrenin* (1: 100,000) into left femoral vein.
11.51 a.m. C. T.—30"—30".
11.54 a.m. Specimen no. 3–4.5 cc.
12.15 p.m. C. T.—2'30"—3'.
12.17 p.m. Specimen no. 4—4.5 cc.

Autopsy shows both nerves cut.

Prothrombin

									Optimum	*Check*
5 gtts.	plasma	no. 1	+2	gtts.	$CaCl_2$	Clotting	time	3'		
"	"	"	3	"	"	"	"	4'		
"	"	"	4	"	"	"	"	4'		
"	"	"	5	"	"	"	"	3'30"		
"	"	"	6	"	"	"	"	3'30"	3'	3'15"
"	"	no. 2	2	"	"	"	"	3'		
"	"	"	3	"	"	"	"	2'30"		
"	"	"	4	"	"	"	"	2'30"		
"	"	"	5	"	"	"	"	2'30"		
"	"	"	6	"	"	"	"	2'30"	2'30	2'15"
"	"	no. 3	2	"	"	"	"	3'15"		
"	"	"	3	"	"	"	"	2'30"		
"	"	"	4	"	"	"	"	2'30"		
"	"	"	5	"	"	"	"	2'		
"	"	"	6	"	"	"	"	1'45"	1'45"	1'45"
"	"	no. 4	2	"	"	"	"	4'15"		
"	"	"	3	"	"	"	"	3'45"		
"	"	"	4	"	"	"	"	3'15"		
"	"	"	5	"	"	"	"	2'45"		
"	"	"	6	"	"	"	"	2'30"	2'30"	2'30"

Experiment XVIII. February 6, 1916. Cat 77. Weight 2.4 k. Both splanchnics cut November 10, 1915.

10.55 a.m. Pithed.
11.36 a.m. Specimen no. 1—10 cc.
11.48 a.m. 0.35 cc. adrenin (1: 100,000) into left femoral vein.
11.57 a.m. Specimen no. 2—8 cc.
12.34 p.m. Specimen no. 3—10 cc.

	SPECIMEN NO. 1 (6 cc.)	SPECIMEN NO. 2 (5 cc.)	SPECIMEN NO. 3 (7 cc.)
Weight crucible + paper + fibrinogen	13.367	13.973	13.5685
Weight cruc. + paper	*13.362*	*13.972*	*13.5680*
Weight fibrinogen	0.005	0.001	0.0005
Fibrinogen per 100 cc. plasma	0.083	0.020	0.007

The graphic representation of the results was made on a percentile basis, with the values obtained in the first specimen of blood taken as 100. For instance in Experiment VIII the prothrombin times are as follows: 4′45″, 2″50″, 3′15″, 3′30″. If 4′45″ is taken to represent the normal amount of prothrombin in the circulating blood, a decrease of prothrombin time to 2′50″ indicates according to Howell an increase in prothrombin provided the antithrombin does not vary. To express this in per cent we call 4′45″, 100 per cent. Then the relative amounts of prothrombin will be in inverse ratio to the "prothrombin times" (inasmuch as the antithrombin does not change) and the results from which the diagram of this experiment would be made would be expressed by the proportion:

$$285'': 170'' : : x : 100$$

$$x = 168 \text{ (the nearest whole number)}$$

Thus, in the experiment, nine minutes after the injection of adrenin there was an increase of 68 per cent in the prothrombin in the circulating blood. A similar procedure was followed in calculating the antithrombin, coagulation time, and fibrinogen values on a percentile basis.

The time of each specimen was noted, taking the time of pithing as the starting point. That is, each specimen is said to have been taken so many minutes after pithing, The times of injection were noted in the same way. These varied somewhat from experiment to experiment but as the same general procedure was followed this variation was not excessive. The times that appear on the charts represent the average time after pithing when the various specimens were taken. There were usually four, occasionally five, specimens. Injections were made after the first specimen, as a rule. When two injections were made they came between the first and second and fourth and fifth specimens of blood for analysis. In averaging the coagulation times those nearest each specimen (the C. T. was done in relation to each specimen of blood for analysis) were arranged and plotted on the chart on the same ordinate as the specimen with which they were connected. The curves themselves scarcely need discussion. From figure 1 it is seen that the decrease in coagulation time is exactly proportional to the increase in prothrombin, while the antithrombin remains a straight line. Unexpectedly it was found in two experiments that the fibrinogen fell, but as was pointed out above, such minute quantities were available that these figures possess very little significance. Whether the low values obtained for fibrinogen in these animals was due to the splanchnectomy must be determined by future work. Figure 2 shows the values obtained in the controls. As will be readily seen, there is practically no change in any of the factors.

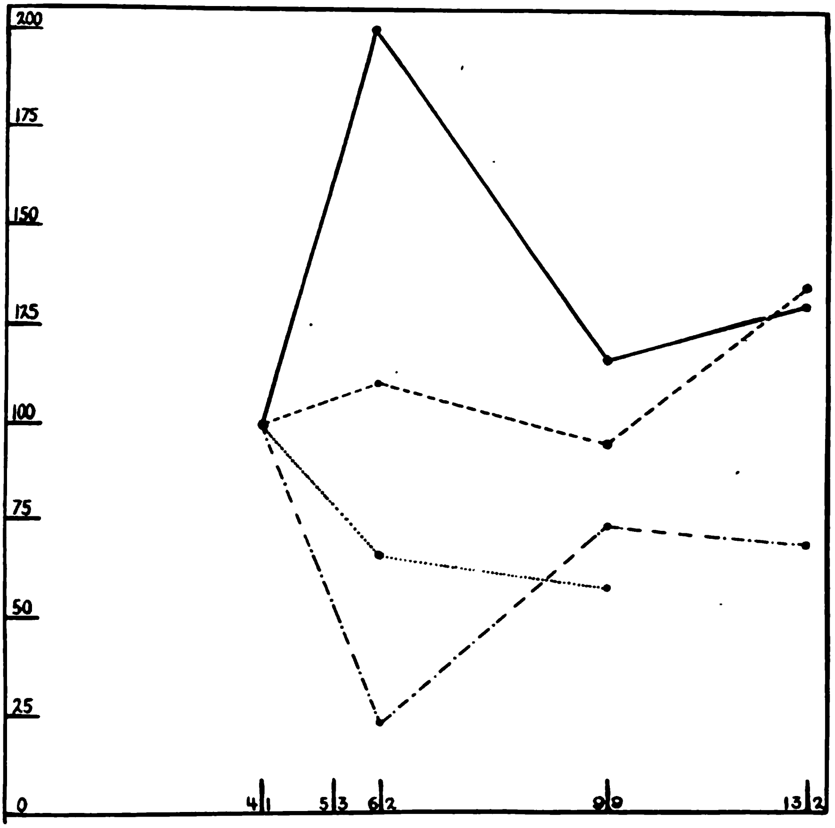

Fig. 1. Solid line represents prothrombin, dash line antithrombin, dotted line fibrinogen, dash-dot line coagulation time. Figures on the ordinate express per cent, those on the abcissa minutes after pithing. The intersection of the ordinate and abscissa indicates the time of pithing. At fifty-three minutes after pithing 0.15 cc. of adrenin (1: 100,000) per kilo was injected intravenously. These curves represent the average of Experiments vii, viii, x, xi and xiii. This figure is the graphic expression of the average percentile variation in these factors after the injection of minimal doses of adrenin. The amount in the blood of the animals before the injection was made is considered to be 100 per cent.

In comparing our results with those of Cannon and Gray it seems likely that prothrombin is elaborated in the liver and possibly in the intestines also (2). In connection with the other actions of adrenin of biological importance, the present fact is of interest taken in conjunction with the fact that hemorrhage decreases coagulation time by decreasing the amount of antithrombin in the circulating blood (6). Under the stress of the major emotions there is a discharge of adrenin which causes a decrease in the coagulation time and under the conditions of savage life hemorrhage is often an accompaniment of such emotions. In such a case there would be a change in two factors of the coagulation time of the blood, each change tending to decrease this time—a powerful mechanism of vital importance to the individual.

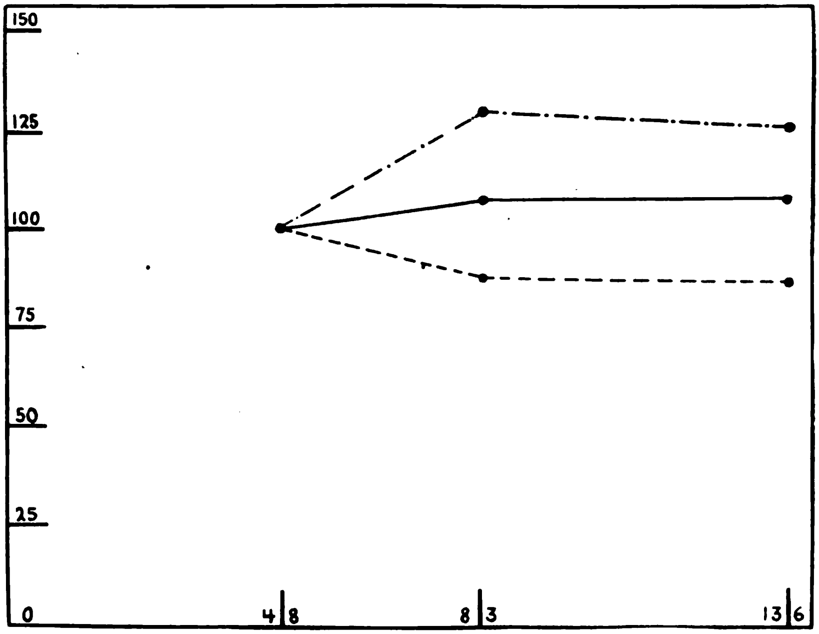

Fig. 2. Solid line represents prothrombin, dash line antithrombin, dash-dot line coagulation time. The ordinate is graduated in per cent and the abscissa measures minutes after pithing. These curves record the average variations in the factors charted of experiments iii, v, vi, xii, xiv, and xv. This figure shows the results obtained in the control experiments in some of which no injection was made and in some of which normal saline solution was injected intravenously.

I wish to express my sincerest thanks to Dr. W. B. Cannon at whose suggestion and under whose guidance the work was done.

CONCLUSION

The intravenous administration of minimal doses of adrenin (0.143 cc. of a 1: 100,000 solution per kilo) decreases coagulation time by increasing the amount of prothrombin in the circulating blood.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXXVI. THE PHYSIOLOGY OF THE GASTRIC HUNGER CONTRACTIONS IN THE AMPHIBIA AND THE REPTILIA. COMPARATIVE STUDIES. I[1]

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INTRODUCTION

It is now evident from the more recent work of Cannon (1) and Carlson (2) that the sensation of hunger is due to contractions of the stomach wall in contra-distinction to the older hypotheses in existence; *e.g.*, the theory that hunger is a general bodily sensation, that it is due merely to emptiness of the stomach, that it arises from hydrochloric acid in the empty stomach, and lastly, that it is due to turgescence of the gastric glands—"turgescence theory" of Beaumont. Notwithstanding the large amount of literature upon the movements of the gastric motor mechanism, little has been done to establish the exact analogy of the gastric hunger contractions throughout the vertebrate series. Certain facts have been ascertained regarding the state of the fasting stomach in higher animals, and certain inferences have been drawn from these facts, but since the complex functions of man are unravelled many times from a study of lower forms it might be of some physiological importance if a series of similar investigations were conducted on the remaining classes of the vertebrates and even perhaps upon certain forms of the invertebrates. Up to the present time, however, all the principal work has been done on a single animal group, the mammalia, with the exception of some work now in progress on the group of the aves. Therefore, in descending the vertebrate scale to new fields of investigation in this particular phase of physiology the next groups in order are the reptilia and the amphibia, respectively. Representatives from these two groups will be dealt with in this report.

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[1] A preliminary report of this work was made before the 1915 meeting of the American Physiological Society at Boston, a brief abstract of which was published in the Proceedings of that society. Also some of the results were presented in a paper read before the Kingston Medical and Surgical Society, February 28, 1916.

Various investigators have worked with the spontaneous movements of the gastro-neuro-muscular apparatus of poikilothermic animals which has been confined principally to the frog under varying conditions. They have studied the excitatory and the inhibitory influences upon gastric motility through its extrinsic innervation by means of electrical, mechanical and chemical stimuli, as well as the direct effects on this organ. With regard to the gastro-inhibitory effect of the vagus certain experiments upon the frog are of special interest, Volkmann (3) in 1841 found that if the medulla oblongata is destroyed or the vagi are cut, a lively peristaltic movement in the stomach is observed. Goltz (4) in 1872 confirmed the above results, but neither of these authors was able to correctly interpret his findings; the former could not yet think of inhibition, and the latter although the interpretation suggested itself could not conceive of the idea that inhibitory and motor-nerve fibers could be contained in the same nerve trunk. The appearance of a movement after the section of the vagi, in a stomach previously at rest, seems to indicate that in a normal state these nerves exert an inhibitory influence upon the stomach. The results obtained by Goltz (5) from curarized frogs which had been left unfed for some days previous to the beginning of the experiment showed in addition, that the automatic movements of the frog's stomach disappeared after the destruction of the brain and cord, as well as after the cutting of both vagi. According to this observation the vagi would contain inhibitory fibers for the stomach, but, since the gastric wall remained in a more or less tonic condition and was not followed by expansion as far as I am able to determine from a review of Goltz's paper, the movements of the frog's stomach itself must have been suppressed by the artificial excitement of the vagi. Furthermore, he states that the mere cutting of the vagus with scissors in close proximity to the esophagus gives rise to violent movements in the wall of the stomach. This would indicate the existence of motor fibers for the frog's stomach. The presence of gastro-inhibitory fibers in the vagi have been conclusively shown by Langley (6), Meltzer (7), May (8), and Cannon (9) in mammals. According to Kronecker and Meltzer (10) the tonus of the cardia for the rabbit at least is diminished or inhibited at the onset of each deglutition, and if many deglutitions follow one another in rapid succession, the tonus may be entirely abolished. This relaxation of the cardia is of central nervous origin since it takes place even when the esophagus is severed, but disappears when the vagi are cut. Auer (11) has reported that only a slight degree of reflex inhibition of the stomach is obtained through the vagi in rabbits. Von Openchowski (12) has described nerve twigs of the vagi in the proximity of the cardia, from one of which he obtained by stimulation contraction, and from another relaxation of the cardia. According to May (8, 13) the vagi contain both motor and inhibitory fibers for the stomach musculature. Stimulation of the vagus produces two effects, namely, an inhibitory or dilatatory effect followed by an increase of gastric muscular tone and movements, the former predominating in the cardiac end of the stomach and the latter in the pyloric end. Similar effects were produced by "anemia" of the stomach when it was suddenly produced. This author also believed that the splanchnics had no direct influence either motor or inhibitory on the stomach muscular wall. Later investigations by Cannon and Murphy (14) and Auer (11) have shown that the inhibition of the gastric movements of digestion is produced through the splanchnic nerves and the same has been verified by Carlson (15) for the gastric hunger contractions. Furthermore, it has been shown by Cannon (16) for the digestive movements and by Carlson (15) for the hunger movements that the gastric tonus is largely maintained through the vagi. Mayer (17) has described three motor effects resulting from vagus excitation in the neck of rabbits, namely, (*a*) The cardial part of the stomach becomes very stiff, then follows a subsequent contraction-pyloric wave. (*b*) The usually observed depression between the cardial and pyloric portions becomes more manifest and depressions in other parts are noted. (*c*) The whole stomach musculature passes into a state of tetanic contraction which persists a long time. Longet (18) did not obtain such results from vagus excitation in the neck of this animal. The discrepancy in the results was due to the fact that in the period of digestion the above mentioned effect of the nerve action is not manifested. Adrian (19) verified by electric excitation of the plexus coeliacus and its neighborhood, movements in the cardia and pylorus.

The experimental work in connection with the excitatory and inhibitory movements of the frog's stomach has not been confined alone to the vagus. Various other nerves under stimulation have been found to exert a marked influence on the gastric motor mechanism. Waters

(20) noticed that strong stimulation of the third, fourth, fifth and sixth nerves in the frog at their exit from the spinal cord led to contractions of the stomach, as well as the esophagus and sometimes the small intestine. Steinach and Wiener (21) have described motor effects in the frog's viscera as a result of stimulation of the posterior roots. According to these authors the stomach is innervated from the third, fourth and fifth spinal nerves. Dixon (22) verified the above results by stimulation of the rami communicantes of the third, fourth and fifth spinal nerves which corresponds to the origin from the spinal nerves described by Waters and Steinach and Wiener. According to this author stimulation of the rami communicantes of the fourth spinal nerve produces tonic contraction together with augmented and more regular automatic stomach contractions. Stimulation of the third and fifth rami produces a similar but smaller effect. The vagus contains inhibitory fibers to tonus but upon excitation of this nerve augmented automatic movements usually result, but no increase in gastric tonus is ever observed. Contejean (23) in studying the innervation of the stomach of Batracians found that electrical excitation of the sympathetic caused no relaxation of the gastric reservoir, it even caused contraction although to a lesser degree than excitation of the vagus. It is with the vagus and not with the sympathetic that this author has been able to provoke arrest phenomena in the peristaltic movements of the stomach, and he thinks the vagus acts especially on the longitudinal fibers while the sympathetic commands the circular fibers although they are probably not absolutely dependent upon the sympathetic. He has also quite regularly provoked contraction of the pylorus in dogs by electrization of the vagus. Meltzer (24) found that very weak excitation when applied directly to the stomachs of dogs, cats, rabbits and frogs caused contraction, if the electrode was placed on the outer side of the stomach. This varied somewhat with the animal used, but the main excitatory parts according to him were the pylorus, the cardia and slightly the fundus, except in the case of the frog in which these points were not definitely localized. Battelli (25) studied the gastric movements under the influence of drugs, and draws the interesting conclusion that the vagus by the inner branch of the accessorius not only regulates the moving phases but also the inhibition phases of the stomach muscles. Doyon (26) found that excitation of the peripheral end of the vagus under ordinary conditions in dogs undergoing digestion, the nerve of the opposite side being intact, invariably provoked exaggeration of the stomach movements, but he

never observed any clearly marked suspensory action of the nerve on the gastric reservoir or a diminution of the gastric tonus. According to this author an injection of pilocarpine or strychnine appears to favor the putting into play of the inhibitory power of the vagus nerve on the movements of the stomach, since excitation of the peripheral end of this nerve after the injection causes a decontraction of the gastric reservoir followed by an exceptionally energetic contraction and he suggests that these substances provoke a combined effect through the gastro-inhibitory and gastro-motor fibers of the vagus. Barbéra (27) used the stomach sack method in investigating the spontaneous activity of the frog's stomach. The brain was separated from the medulla and two cannulas were introduced into the stomach; one into the cardia and held by an esophageal ligature, and the other through the pylorus and fixed by a duodenal ligature. The stomach was then filled with normal saline solution at a small pressure of from 3 to 4 cm. in the ascending cardial orifice and connected with a Marey apparatus for registering the movements or alternations in its internal capacity. With such an arrangement Barbéra studied the effect of electrical excitation on the gastro-spontaneous movements, and concluded that the stomach muscular movements were reflex movements. Gläfsner (28) with a somewhat similar arrangement studied the effect of different substances (poisons) in the stomach. He used a double cannula instead of two single cannulas which he introduced through the esophagus. Dixon (22) undertook a similar inquiry but used an internal pressure of from 10 to 20 cm. up in a frog's stomach. He thought that the optimal water pressure varied greatly with different animals and emphasized the fact, that the spontaneous movement with his method exhibited greater regularity.

There were generally differences of opinion as regards the animals among authors. Some thought that regularity and strength of the spontaneous contractions varied according to the animal, and that in the case of frogs, winter frogs were not suitable. Also adverse ideas existed as regards unfed and fattened frogs. Morishima and Fujitani (29) investigated the spontaneous movements of the frog's stomach (Esculenta) with a William's heart apparatus. They isolated the whole stomach in toto, but used only the part in the neighborhood of the pylorus, so that, when the double-walled cannula was tied in the fundus the prepared "Magensack" constituted about one-third of the whole stomach wall. The pyloric end was closed by a ligature and the whole placed into Ringer's solution into which a slow current of oxygen was led. These authors verified that regular and strong contractions are obtained with well fed frogs, and they do not think that the condition of the stomach, that is, full or empty is of great consequence. Gläfsner (28) used either freshly caught hungry frogs, or those confined a long time, fed on rain-worms, etc., and then left without food for some days before the experiment. Dixon (22) thought that hungry frogs such as Gläfsner and others used gave much worse results than normal animals, and that if kept in confinement for two months were quite unfit for use. According to Morishima's and Fujitani's experience the stomach filled with food is the best for studying spontaneous movements, but in case of frogs kept a long time in confinement better results are obtained if frog-muscle or rain-worms are fed them some days before the experiment. More recently, Hopf (30) with a similar arrangement to Barbéra's has shown conclusively that stimulation of the vagus in the frog can have the effects of inhibition and of excitation on the gastric reservoir. In order to obtain good results the stomach must be placed in a good condition of automatic contraction (motility). The phase of inhibition is then seen preceding that of excitation, and the excitation is stronger than the inhibition, the intensity and the duration of the current having little or no influence on the vagus effect. According to this author there is a great distinction between fed and unfed frogs in the size and stability of the automatic stomach movements, but winter frogs give good results if previously fed.

EXPERIMENTAL PROCEDURE

The comparative studies on the amphibia and the reptilia were made on the bullfrog (Rana catesbiana), better known in the fish markets as the "jumbo frog," and the common snapping turtle (Chelydra serpentina), respectively. All the bullfrogs were obtained from the South, Louisiana, through local dealers in Baltimore and Chicago and were stomostomized. This simple operation which has only been briefly described by the author (31) consists of making a circular opening on one side between the ramus of the inferior maxillary near the posterior angulosplenial region and the anterior cornua of the hyoid bone through the skin, the submaxillaris (mylohyoideus) muscle and the lining membrane of the pharyngo-oral cavity (32) of sufficient size (about 8 mm. in diameter) to admit the balloon and the attached rubber tube which connects with the recording manometer. This operation may be bloodlessly performed provided care is taken to avoid injury to the superficial mandibular vein (vena maxillaris inferior) which runs along the insertion of the submaxillaris muscle, and turns inwards at its hinder border to join the lingual vein. Through this artificial opening or stomostomy which usually heals in from three to four days so that the animal may be used for experimental tests with normal results is introduced the delicate rubber balloon by means of a glass seeker. Then by opening the frog's mouth the balloon may be carefully pushed into the stomach with the seeker through the short esophagus which may be greatly enlarged, yet when empty is completely closed by folding of the walls. In this position the balloon may be inflated through a glass T-tube and the desired pressure obtained in the manometer, the rubber tube passing through the stomostomy to which the balloon is attached lying in the posterior part of the pharyngo-oral cavity under the free edge or at the side of the tongue, and thereby eliminating any possibility of the stomach pressure fluctuations on the balloon which are transmitted by air transmission to the surface of the liquid in the manometer being partially or entirely shut off, which would be the case, if the flexible rubber tube passed between the jaws so the animal could bite upon the tube. Like the gastric fistula in dogs there is no trouble in the way of closing up of the stomostomy as long as the animal is being used daily, but when unused it usually closes up completely in from five to seven weeks. The opening appears to be of practically no inconvenience to the animal. It will have to be admitted that practically all the methods previously used by investigators for studying the spontaneous movements of the frog's stomach were more or less pathological in their entirety, while the method used in this investigation allows the animal to remain in a perfectly normal condition at all times, since the stomostomy in the floor of the mouth does not interfere in any way with the normal physiological activity of the gastric motor apparatus or any other system of the body. The large species of frog is also advantageous with this method since more accurate results are usually obtained from larger animals.

During the record taking of the gastric hunger movements, the animals were placed individually in small laboratory table sinks, 7 by 10 inches and 5 inches in depth. The bottoms were covered with several layers of filter paper to partially close the outlet and water was allowed to drip very slowly from the faucet. Each sink was then covered with the exception of a very small opening for the passage of the rubber tube from the balloon in the frog's stomach to the manometer. With this arrangement the animals were practically concealed from all disturbing influences and the sinks, darkened as they were by opaque covers, the animals felt themselves securely hidden and would remain very quiet for long periods of time. Two animals were always run at the same time, the one as a control on the other, and the same tests were always applied to both. For the temperature experiments it was necessary to use different devices. To determine the effects of raising the temperature upon the movements of the gastric hunger apparatus, the animal was placed in a glass cylinder of 7½ inches diameter and 6 inches in height, having a 2¾ inch raised base of wire gauze covered with a circular galvanized iron plate, the size of the interior of the cylinder. The animal stood upon the raised base and occupied the upper 3¼ inches of the cylinder. The top of the cylinder was provided with a wooden cover containing two small openings, one for a thermometer, and the other for the rubber tube from the balloon. Around the glass cylinder was wrapped a dark colored wet towel to darken its interior and the whole was placed in a large vessel containing water at ordinary room temperature to a height that rose within about ½ inch of the galvanized iron plate within the cylinder. The temperature of the water was now very gradually increased at the rate of about 10°C. per three hours and continuous records of the hunger movements recorded. On the other hand, the effects of cooling or lowering the temperature was determined by placing the animal in a box 12 by 10 inches and 8½ inches in height, the sides of which were covered with ¼ inch mesh wire gauze and the top and bottom of wood. Around the sides and top was placed a dark colored wet towel to darken the interior as in the case of the cylinder above, as well as to shut out all possible disturbing influences. The animal so arranged was placed in a refrigerator, the door of which having been previously left open until the temperature of its compartment reached approximately that of the ordinary room temperature. The temperature was obtained by a thermometer in the box which was very gradually lowered by the gradual closing of the refrigerator door at the rate of about 10°C. per three hours, while continuous records were being taken. However, in one case where it was desired to reduce the temperature to a very low degree, a freezing mixture of cracked ice and salt was packed around the glass cylinders above described and then the animals placed in position and connected with the recording apparatus. As might be expected, this did not give nearly as satisfactory results as did the refrigerator method, since the temperature was reduced too rapidly. However, this very low temperature did bring out certain additional facts of rather great importance which the other method did not.

In the species of turtles used the plastron is small and narrow, exposing a great amount of the fleshy parts, therefore it was possible to perform a gastrostomy on these animals. An incision about 3 inches in length was made near the posterior curvature of the plastron of the left side through the abdominal wall, and the ordinary procedure then followed as outlined by Carlson for gastrostomy on dogs (33). As a rule, snapping turtles are very vicious and are rather unique among chelonians in defending themselves in a similar manner to snakes, that is, by "striking" at the object of anger. The rapidity with which the head is shot forward when these animals are disturbed rivals the dexterity of the rattlesnake, and when we consider that they are provided with a pair of keen-edged, cutting mandibles and jaw muscles of tremendous power, the stroke of these dangerous brutes may be followed by anything but superficial injury. In fact the amputation of a finger by a medium-sized specimen, or a hand by a very large individual would be an accomplishment of no difficulty to the reptile (34). Therefore great precaution must be used in handling these animals for experimental purposes or otherwise. The safest way to handle a large specimen is to pick it up by the tail and hold it well off from the body, but I have found it also quite safe to handle it by the posterior lateral portions of the shell. The animals used weighed from 10 to 15 pounds, and the hunger contractions were recorded by a balloon introduced through the fistula into the stomach. During the recording of the contractions the animals were placed in a specially constructed box, 17½ by 10½ inches and 4½ inches in depth and supported on legs 13 inches in height, in the bottom of which was an oval opening 7 inches in length by 4½ inches in width. This opening in the bottom of the box gave access to the fistula for the introduction of the balloon into the stomach and removed all possible danger of injury from the vicious animals. This box was provided with a lid at one end covered with wire gauze and was of such size that the animal was comfortable and could move about slightly, although the internal capacity was not sufficiently large to permit the animal to turn completely around. With this arrangement the animal after it had once quieted down would remain quiet for hours and continuous records of the gastric hunger movements could be obtained with ease. All the records from the frog and turtle were taken on a slowly moving drum revolving at the rate of about fifty minutes per revolution.

THE CHARACTER OF THE GASTRIC HUNGER MOVEMENTS FROM THE EMPTY STOMACH OF THE FROG

The recorded observations upon the activity of the gastric hunger mechanism of the frog have numerous points of dissimilarity as compared to the numerous observations already on record for higher animals. Thus, Carlson (35) has demonstrated that the empty stomach during prolonged fasting exhibits three types of motor activity, namely:

1. Rhythmical contractions of about twenty seconds' duration and designated the "twenty second rhythm."
2. Very strong contractions occurring periodically of about thirty seconds' duration and designated the "thirty second rhythm."
3. Tonus changes of the stomach musculature.

In the frogs under observation there were no tonus changes observed, not even in prolonged fasting. This would seem to confirm the work of Dixon (22) on the vagus of the frog, namely, that excitation of this nerve causes no increase in gastric tonus although augmented automatic movements usually occur. Since the different types of these hunger contractions are dependent upon the degree of tonus of the stomach and since there are no tonus changes in the frog's stomach it would be expected that only one type of hunger contraction would be exhibited by this animal, and this is found to be the case. This particular type of contraction shows an average duration of about one and three-fifths minutes, and the intervals between the contractions vary from sixteen to thirty-three seconds. These contractions are very powerful and are evidently analogous to the thirty seconds rhythm in man and dog, type I contractions, but the individual contractions are very much more vigorous, that is, if we take into consideration the body weight of the frog as compared to the body weight of the above mentioned animals. In fact, they even excel in strength and amplitude, the most powerful contractions from either man or dog in prolonged fasting (fig. 1). In general appearance the contraction is rather slow, the curve is perfectly smooth and shows no smaller superimposed waves, and there is no indication that the contractions fall into groups, separated by intervals of relative quiescence. In the higher animals the hunger contractions are periodic and represent simply more vigorous peristaltic movements during which the stomach becomes markedly hypertonic (40, 52). On the other hand, the gastric hunger contractions of the frog are continuous and show a definite regularity which goes on indefinitely hour after hour and day after day with no periods of rest except for the few seconds' pause between each individual contraction. This corroborates the work of Morishima and Fujitani (29) on the frog (Esculenta) that very regular curve tracings may be taken hourly, eventually ten hours, and they have published a series of these normal curves selected at definite intervals which extend over a period of about eight and a half hours from the beginning of the experiment. This corroboration is all the more valuable, as the method employed by these authors is totally different from the one used by myself. It was also found that when the pressure in the balloon in the frog's stomach was increased that it

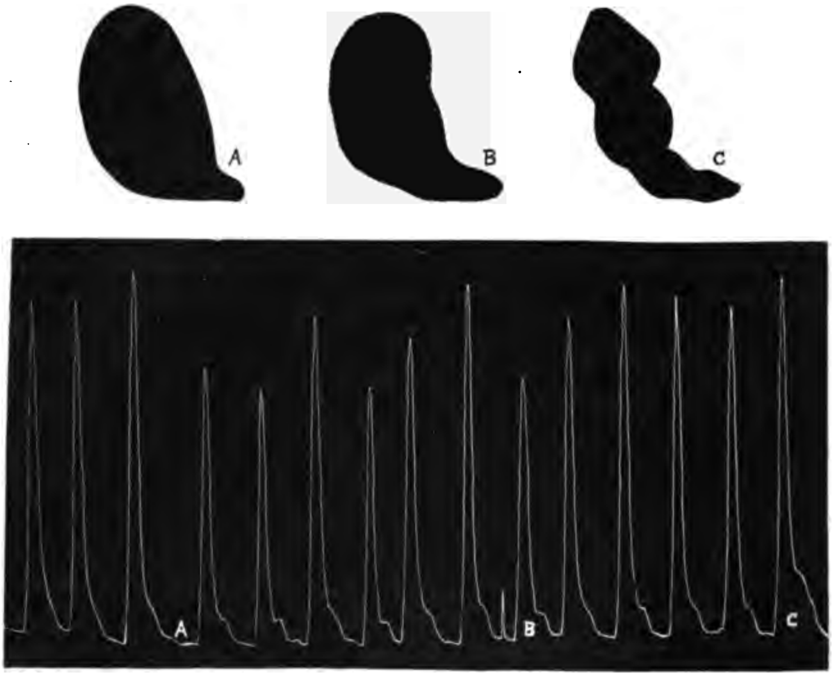

Fig. 1. Hunger contractions of the frog's stomach after twelve days' fast. *A*, outline of stomach (as seen by X-ray and bismuth balloon), between hunger contractions with stomach relaxed. *B*, outline of stomach at the height of a moderately strong hunger contraction. *C*, outline of stomach at the height of a very strong hunger contraction. Water manometer.

increased the pressure in the manometer only momentarily, the stomach simply dilating to accommodate itself to the increased size of the balloon. This may be repeated several times with apparently no change in the contractions or increase in the manometric pressure except temporarily, but finally a limit of stomach expansion will be reached in which to further increase the pressure by blowing reasonably will be impossible and at the same time the manometric pressure will be markedly increased. This no doubt, is an adaptive accommodation which this low form of vertebrate animal possesses, since the periodic habit of feeding is unformed and when food is abundant it eats greedily, the stomach dilating to act as a reservoir for the food.

THE CHARACTER OF THE GASTRIC HUNGER MOVEMENTS FROM THE EMPTY STOMACH OF THE TURTLE

The gastric hunger movements of the turtle's stomach show an advance in physiological development over the gastric hunger activity of the frog, and conform more to the results obtained from the stomachs of higher animals. In fact, this would be the natural thing for us to expect considering the different positions that these two animals occupy in the animal scale. Slight tonus variations are observed and two distinct types of hunger contractions may be described in the turtle. The first type of contraction in this animal (fig. 2, *A*) shows an average duration of about one minute, and the intervals between the contractions vary from three to three and a half minutes. These contractions at first appear to be less vigorous than those from the frog's stomach and perhaps for this reason they are slightly shorter in duration. This type of contraction is characteristic of the early part of the hunger period and is no doubt analogous to the thirty seconds rhythm in man and dog, type I contractions. It also corresponds to the type I contractions described for the frog. The small irregular waves which appear here and there in the rest intervals between the individual contractions are exaggerated respiratory movements. The gastric contraction of the turtle like that of the frog is rather moderate in appearance and the curve is perfectly regular with no indication of smaller waves superimposed upon it. In the latter part of the active hunger period the type I contractions above described gradually change into a second type of contraction which tends to approach incomplete tetanus at every

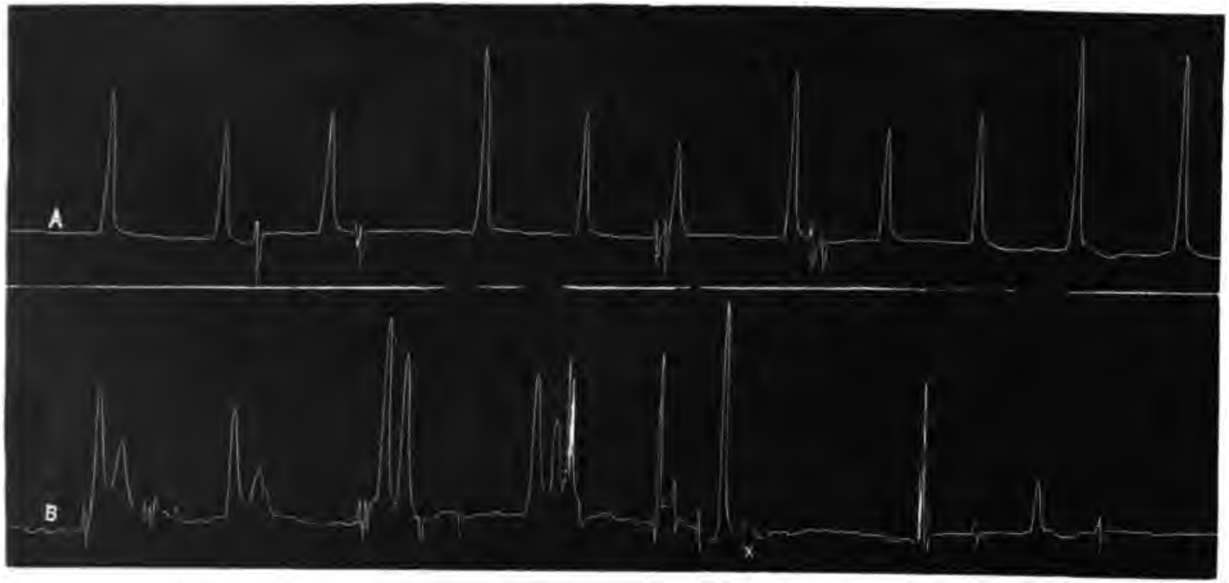

Fig. 2. Hunger contractions of the turtle's stomach after twenty-one days' fast. *A*, initial forty minutes of a five and one-half hours' typical period of hunger contractions. *B*, final thirty minutes of the same active period. At *X* the active period terminates and the stomach passes into a period of quiescence. Note in tracing *B* the incomplete tetanus. Water manometer.

regular periodic contraction of the stomach and this effect becomes more marked as the contractions continue. There is also an increase in gastric tonus during each contraction with its tetany and usually a slight but general increase in the whole tone of the stomach musculature (fig. 2, *B*). This second type of contraction is probably the primitive ancestor of the twenty seconds rhythm in man. The contraction with its incomplete tetanus shows an average duration of about two minutes, and the intervals between the contractions in this case vary from three and a third to four minutes. The hunger contractions of the turtle unlike those of the frog but resembling those of the higher animals fall into groups of gastric activity, separated by intervals of relative quiescence. The duration of the periods of gastric hunger activity vary from five and a half to six hours and the intervening periods of quiescence of the stomach from one to one and three-quarters hours, with but one exception in which the quiescent period was found to be of four and one-sixth hours' duration. The period of gastric hunger activity in the adult turtle appears to be relatively long as compared to that of an adult dog (36) which ranges from one and a half to three hours in length but again when we stop to consider the place of this animal in the vertebrate scale, we would expect it to take an intermediate position and that the gastric activity would also be intermediate between the frog and the higher animals, and this is evidently the case. The gastric motor mechanism of the turtle shows a physiological development above the state of a simple, continuous contracting organ so characteristic of the stomach of the frog, yet it has not become sufficiently developed to be classed physiologically with the stomachs of higher animals, and therefore must play the important part of a connecting link in the gastric motor mechanisms of animals. The stomach of the turtle like that of the frog also exhibits the property of expansion and dilatation as determined by increasing the pressure in the balloon.

THE INHIBITION OF THE GASTRIC HUNGER CONTRACTIONS IN THE FROG AND THE TURTLE

It is now demonstrated that the tonus and the hunger contractions of the empty stomach in man and dog are temporarily inhibited by mechanical and chemical stimulation of the nerve endings in the mucous membrane of the mouth, in the esophagus, and in the gastric mucosa (37). Similar stimulation when applied to the intestinal mucosa also produces the effect of gastric inhibition (38). This inhibition is initiated by stimulation of nerve endings in the gastric mucosa, and not by mechanical tension or pressure on the stomach wall. The results on normal frogs compare generally with those on man and dog already reported with some few exceptions. When water, sodium carbonate—1 per cent solution and hydrochloric acid—0.5 per cent solution are introduced very slowly into the empty stomach of the frog, through a small rubber tube passing through the stomostomy, they invariably produce inhibition varying in degree with the stimulating power of the substance introduced. The duration of the inhibition depends upon the quantity and nature of the material introduced into the stomach and not so much upon the degree of the hunger contractions as in the case of the higher animals, since there are no tonus changes and only one type of contraction exhibited by this animal. In normal frogs 5 to 10 cc. water introduced directly into the stomach via tube usually produces a weakening of the contractions (fig. 3, *A*) but never in any of my experiments have I witnessed complete inhibition of the gastric movements. The partial inhibition is seen to come on very gradually as indicated by a lessening in the amplitude of the hunger contractions which usually extend over a period of from ten to fifteen minutes followed by a gradual recovery to normal. The water so introduced was at ordinary room temperature and should correspond approximately to the temperature of the frog's body, therefore the water in the stomach must have produced the temporary inhibition through a stimulation of the nerve endings in the gastric mucosa either by mechanical pressure or by osmosis. According to this view, the passage of the water out of the stomach into the intestine, or the addition of sufficient salts to prevent stimulation by hypotonicity would mark the cessation of the inhibition. It was also found that the inhibitory action of cold water was slightly greater than that of water at the temperature of the frog's body, but in order to bring out this phenomenon effectively it was necessary to use very cold water, which evidently stimulated the protopathic temperature nerve endings in addition to those acted upon by pressure and osmosis. The results overbalance any error that might be accounted for by the cold water cooling the air in the balloon and thereby temporarily lowering the tension in a very small degree. The introduction of 5 cc. sodium carbonate, 1 per cent solution directly into the stomach produces a more marked inhibitory effect than does the water, the contractions may be greatly weakened or they may be completely inhibited for one and a half to two minutes (fig. 3, *B*). Five cubic centimeters hydrochloric acid, 0.5 per cent solution causes complete inhibition of the hunger contractions for periods ranging from seventeen to twenty-five minutes (fig. 3, *C*). The duration of the inhibition in the case of acids and alkalies seems to be directly proportional to their concentration and the total

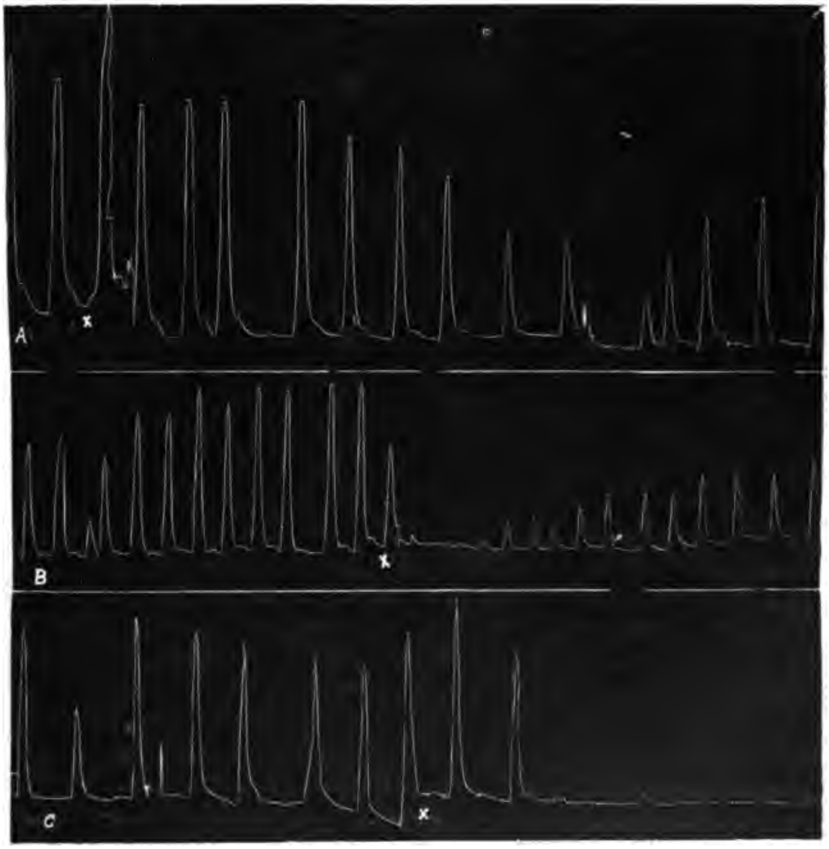

Fig. 3. Records from the frog's stomach after fourteen days' fast showing the inhibitory effects produced by different substances when introduced into the stomach. *Tracing A:* At *X* 10 cc. water introduced directly into the stomach. (The slight drop in tonus after *X* was due to bubbles of air escaping through the manometer.) *Tracing B:* At *X* 5 cc. of 1 per cent solution sodium carbonate introduced directly into the stomach. *Tracing C:* At *X* 5 cc. of 0.5 per cent solution hydrochloric acid introduced directly into the stomach. Water manometer.

quantity introduced, it being the most marked always in the case of the acid. The cessation of the inhibition by these substances probably marks the passage of the same into the intestine or their fixation and neutralization. The inhibition of the gastric contractions in the frog varies from that of the dog and man in that they are not immediately produced after the introduction of the substance into the stomach, but there is usually from one to four or five strong contractions before the inhibition becomes complete. This would seem to indicate that the gastro-reflex apparatus of the frog had not reached the high degree of efficiency found in the stomachs of higher animals. The above mentioned substances when introduced directly into the stomach of the turtle via small rubber tube through the fistula produced the same general inhibitory effects as in the frog.

When water, alkali or acid of the above concentration and in amounts as used for the stomach were introduced directly into the mouth cavity of the frog through a small rubber tube which just passed through the stomostomy into the mouth it was found that the inhibitory effects produced were very slight and perhaps might even have been negative altogether were it not for the fact that some of the solution probably finds its way immediately to the stomach and there may produce inhibition by coming in contact with the gastric mucosa. These observations on the difficulty of inhibiting the hunger movements from the mouth are in agreement with those reported by Rogers (39) for the rabbit and by King (40) for the guinea-pig, except in these two animals the reflex inhibitory mechanism of the stomach from the mucous membrane of the mouth appears to be entirely absent, since these authors have obtained only negative results. The above mentioned substances when placed into the mouths of higher animals (dog and man) produces a marked inhibition of the gastric hunger movements reflexly, therefore it would be reasonable to believe that the cerebral processes are not as highly developed in these animals, the frog included as in the case of man and the dog. It was impossible to try any of these experiments on the turtles because of the viciousness of these animals. Reflex or psychic inhibition of the hunger contractions in both the frog and the turtle was obtained by showing these animals a small live grass frog on a covered glass plate. This agrees with the results obtained from dogs that the sight or smell of food leads to temporary inhibition of the gastric hunger contractions. Anything which frightens, annoys or angers these animals also leads to temporary inhibition of the contractions.

THE MOVEMENTS OF THE FILLED STOMACH AS COMPARED TO THE HUNGER MOVEMENTS AND THEIR INHIBITION IN THE FROG

In the higher animals (dog and man) there is a marked distinction between the gastric movements in normal digestion and the gastric hunger movements of the empty stomach. Acids in concentrations equal to that of the gastric juice when introduced directly into the stomach do not inhibit the movements of the stomach in digestion but will inhibit the hunger contractions (41). In the rabbit (39) and the guinea-pig (40) these two types of gastric contractions are less sharply differentiated, although during fasting the stomach activities may be greatly augmented. In the case of the rabbit the inhibitory action of acid gives the same general results as for the higher animals, with the exception that the effect appears to be less pronounced. In the guinea-pig, according to King, acid placed in the stomach gives negative results.

Since the cannibalistic traits of the bullfrog are well known (42) it is a very easy matter to obtain these animals with a filled stomach, and furthermore, if the animals are kept in separate compartments, the amount of food can be controlled at any one feeding. The animal when hungry usually captures its prey (small frog) by the anterior portion of the body as soon as it strikes the water and swallows it alive, and I have observed one of these animals several times to devour as many as four adult grass frogs in as many minutes and have watched with intense interest the struggling of the smaller frogs in the stomach of the larger. At times they would appear to crawl up into the esophagus when it would become necessary for the larger frog to swallow vigorously several times in order to keep them down. However, these animals are soon asphyxiated since the large esophagus is completely closed by foldings of its substance and therefore effectually prevents any accession of air into the stomach. The frogs for the experiments were usually fed two live grass frogs each, and after a period of from thirty minutes to one hour in order to allow sufficient time for asphyxiation the balloon was introduced into the stomach and connected with the recording apparatus. Records of the frog shortly after feeding (fig. 4, *A*) when compared with the normal hunger records (fig. 1) show but very little change, if any, from the stomach of the hungry animal, the only observable variation being perhaps a very slight increase in the rate of the contractions. This is in confirmation with the view put forth by Morishima and Fujitani (29) that the stomach, full or empty, is not of great consequence as regards the regularity and intensity of the spontaneous movements, although they did not offer sufficient proof to uphold their hypothesis. These facts are contradictory to the ideas of Hopf (30), who claims that there is a great distinction between fed and unfed frogs in the size and stability of the automatic stomach movements, and it is probable that the method which he employed was partly at fault and pathological.

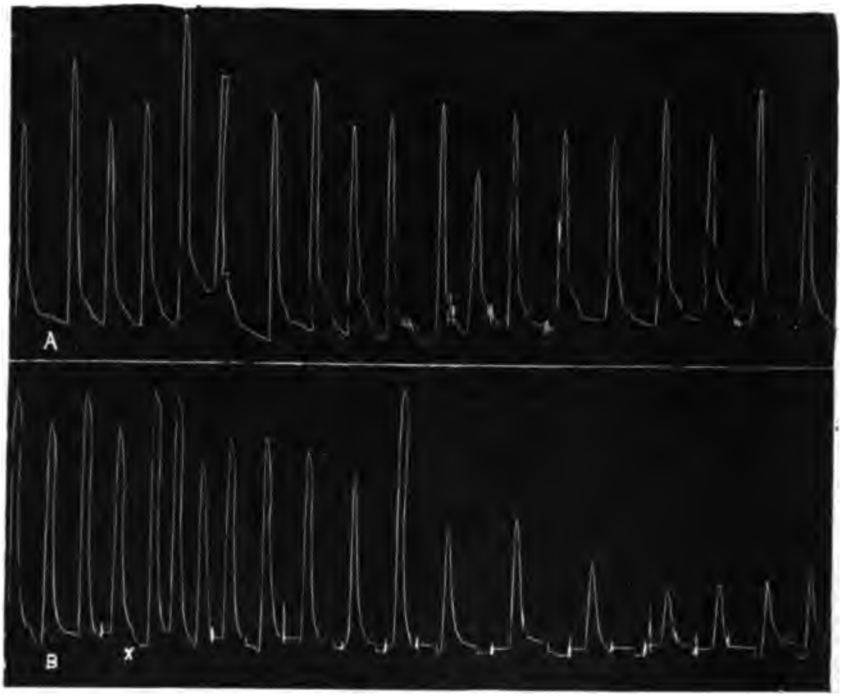

Fig. 4. Normal digestive peristalsis of frog's stomach. *A*, 3 hours after feeding two small live grass frogs. *B*, same animal 10 hours after feeding, food still in stomach. At *X* 10 cc. water introduced directly into stomach producing temporary inhibition. Water manometer.

The question arose in respect to how long it required for the stomach to empty itself after feeding. This was determined after feeding the "jumbo" one small frog and then examining from time to time the stomach contents by inserting a pair of forceps through the mouth and esophagus into the stomach and exposing the digesting animal. I was also interested to find in reviewing the literature after completing the experiments that this same method was used by John R. Young (43) of Maryland in 1803 in determining the rate of gastric digestion in the frog as a part of a medical dissertation submitted to the University of Pennsylvania. However, after some experience I could determine by palpating over the region of the stomach as to its condition and I found by both of these methods that it required from forty-eight to sixty-eight hours for the stomach to completely empty itself. With larger feedings of two, three and four small frogs, the stomach would evidently require a longer period to empty itself. Young did not determine the total time necessary for the stomach to completely empty itself, he simply studied the rate of digestion. In regard to the introduction of water, alkali or acid it was found that they produced similar inhibitory effects in both the hungry and the filled stomach of the frog (fig. 4, *B*) whereas in the case of the higher animals, these same substances inhibit the hunger contractions but have no effect upon the gastric peristalsis with but one exception, namely, in the guinea-pig in which these substances have no effect whatever on either the hunger or the peristaltic movements. This is a little out of harmony with the results obtained from other animals and one would naturally expect from a comparative standpoint that this animal from the position it occupies in the animal scale should possess a reflex inhibitory mechanism from the gastric mucosa. King (40) states that she had difficulty in introducing substances directly into the stomach but in a few instances succeeded in introducing about 1 cc. of the various substances. Had she introduced more of these substances would she not have brought out the reflex inhibitory mechanism of this animal? From the above results it would appear that in the frog, at least, we have a much simplified gastric mechanism which through the processes of evolution has in the higher animals differentiated into the gastric digestive peristalses and the gastric hunger contractions, the latter of which perhaps may be described as intensified gastric digestive peristalses. Thus, the automatism of the gastric mechanism of the various classes of vertebrates apparently stands in correlation with the degree of development and influence of the central nervous system.

THE EFFECT OF PROLONGED FASTING ON THE GASTRIC HUNGER MOVEMENTS

In prolonged fasting the stomach activities in all mammals so far studied are greatly augmented and show without exception marked tonus variations in the stomach musculature. In dogs during pro-

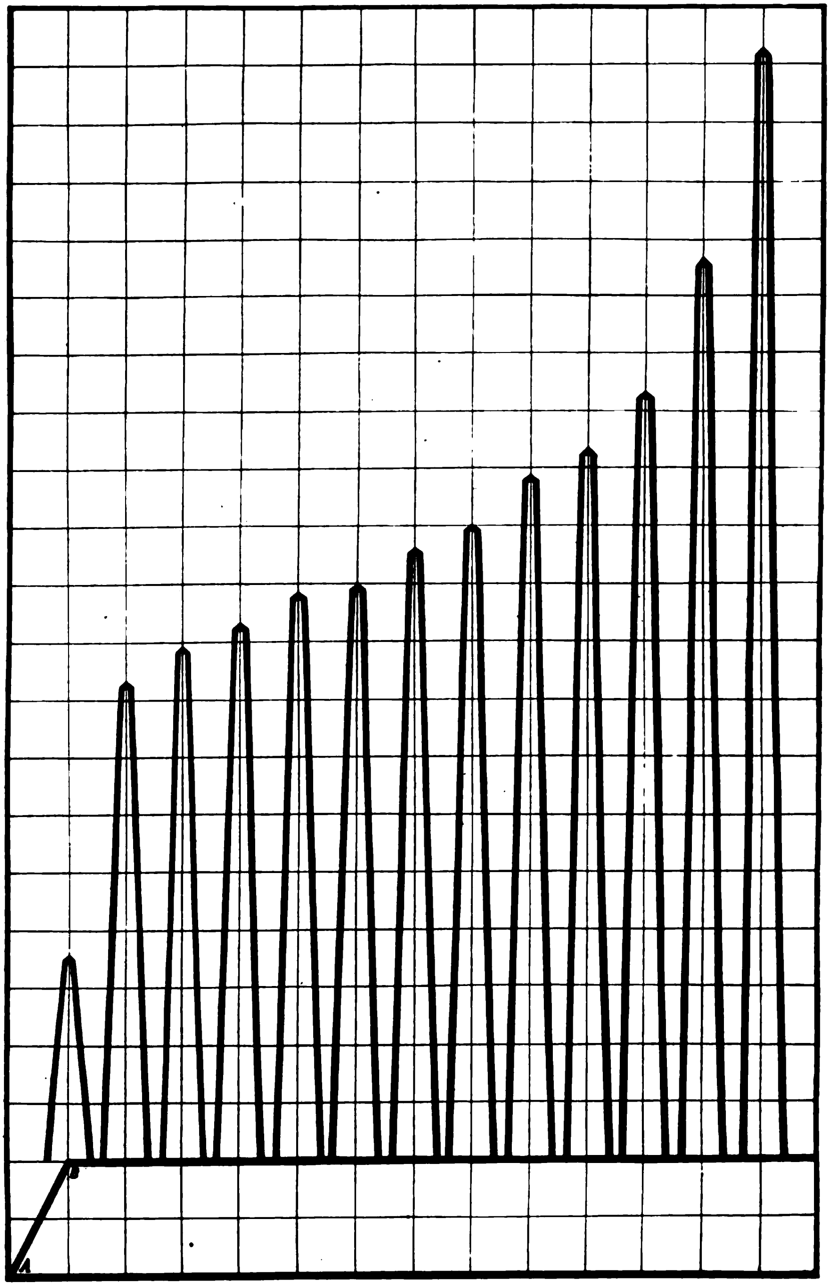

Fig. 5. Diagrammatic representation of the increased amplitude of contraction on the base of the constant tonus as constructed from the tracings of the turtle on every fifth day. Each of the above squares represents one sq. cm. The erect pyramids indicate the amplitude of the hunger contractions in centimeters arranged on the constant tonus as a base line. Spaces left to right indicate periods of five days' fasting. Heavy line at bottom of chart = 0 mm. pressure of water manometer. *A* to *B* = constant pressure of 2 cm. used throughout the experimentation. Note the enormous increase in the amplitude of the hunger contractions at the end of the 64 days' fast.

longed fasting there is a marked increase in the gastric tonus to within a few hours of the death of the animal, or at least, until that point is reached where the stomach becomes involved in the general debility and cachexia, and this increase in the gastric tonus appears to be directly proportional to the decrease in the amplitudes of the hunger contractions (44). In the turtle there are only slight tonus variations while in the frog there are no tonus variations of sufficient strength to be recorded by my apparatus. There is no general increase in the gastric tonus in the turtle in prolonged fasting but we have instead a marked increase in the amplitudes of the hunger contractions with no appreciable increase in the gastric tonus as is shown by the accompanying chart (fig. 5). The same results are indicated in the frog although this animal was not subjected to such long periods of fasting. These results are just the reverse of what is found in the case of the dog. The longest fast on the turtle covered a period of sixty-four days, a little over nine weeks, during which time the animal took no food but had access to water except while the stomach records were actually being recorded. However, in the latter part of this fast there probably entered into the work to some extent the factor of thirst, since it was found advisable not to change the animal too often from the specially constructed box as the animal at this stage of the fast had become very restless and any disturbance tended to increase the restlessness, which made the record taking very difficult. At about the beginning of the fifth or sixth week of the fast, the turtle became so restless and uneasy that a good tracing could hardly be obtained. This restlessness gradually increased in intensity until about the eighth week, then gradually began to subside until the end of the fast when the animal had become quite passive again. This indicates that the disagreeable hunger pangs in the turtle probably do not start as early as in the higher animals (45) and that it also requires a longer time to fatigue the neurones in the central nervous system which have to do with the sensation of hunger. This is the result which we would rather expect in this low form of vertebrate since periods of enforced starvation are not uncommon among wild animals. The amplitudes of the hunger contractions gradually increased with the length of the fast, the contractions became longer and the rate slower. The contractions after a nine weeks' fast (fig. 6) show an average duration of about four minutes and the intervals between the contractions vary from three and a half to four minutes. It will be seen from the above that the length of the hunger contractions under these conditions have been increased from two to four times. It is also of interest to note of the ease with which these enormous hunger contractions are inhibited by forced respiratory movements even at the time of the onset of one of these very strong contractions as in (fig. 6, *B*). These respiratory movements or sighs are also exaggerated in prolonged fasting and during each the animal usually gives a hissing sound.

THE INFLUENCE OF TEMPERATURE ON THE GASTRIC HUNGER ACTIVITY, SEASONAL VARIATIONS AND HIBERNATION

The data collected under this head indicates that the effects of temperature on the gastric hunger mechanism of the frog must be considered under two heads: First, the accelerating action of increased temperature on the chemical processes involved; and second, the inhibitive or retarding action of very high or very low temperatures. Biologic phenomena are undoubtedly largely the result of chemical reactions going on within the living substance, and consequently we would expect that the well-known rule of van't Hoff and Arrhenius concerning the velocity of chemical reactions in relation to temperature, should apply, that is, for each increase of 10°C. in temperature the velocity of a reaction is doubled or trebled. Thus, the above rule within certain

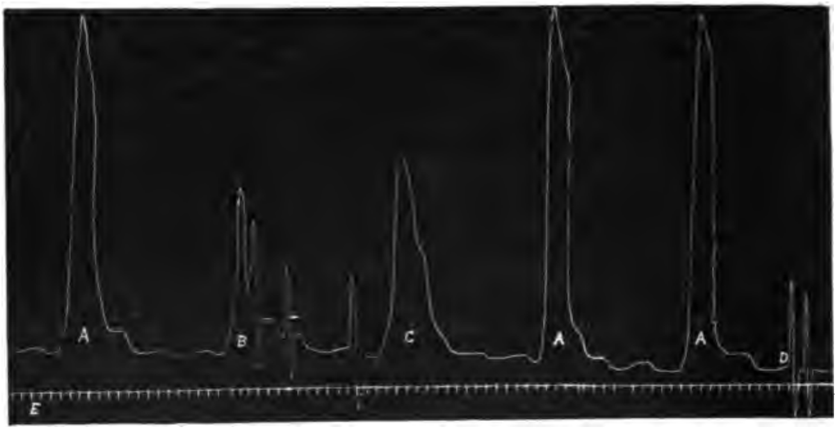

Fig. 6. Hunger contractions of the turtle's stomach after 9 weeks' fast. *A*, normal contraction. *B*, inhibition of the very strong hunger contraction by the respiratory movement, *D*, with recovery through *C*. *E* = time in 30 second intervals. Water manometer.

limits has been verified by Riddle (46) on the process of digestion in cold-blooded vertebrates, by Rogers (47) and Galeotti and Piccinini (48) on the rate of the heart beat for vertebrates and invertebrates, by De Bonis and Midulla (49) for the movements of the frog's stomach by the stomach ring method, and for many other physiological activities.

Tables 1 and 2 show the results obtained from the experiments with the number of contractions executed in the unit of time. The temperature coefficients are obtained as the quotients resulting from the formula

$$\frac{\text{Rate at } Tn}{\text{Rate at } T \times \frac{x}{10}}$$

Tn being the higher temperature, T the lower temperature, and x the difference between these two.

This is the formula used by Riddle.

TABLE 1

Raising the temperature

NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS—ROOM TEMPERATURE	NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS AFTER INCREASE OF TEMPERATURE	TEMPERATURE COEFFICIENTS
23.5° = rate of 5	32.5° = rate of 9	2.00
25° = rate of 6	32.75° = rate of 10	2.15
24.5° = rate of 6	32.25° = rate of 10	2.15

TABLE 2

Lowering the temperature

NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS—ROOM TEMPERATURE	NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS AFTER DECREASE OF TEMPERATURE	TEMPERATURE COEFFICIENTS
20° = rate of 7	14° = rate of 5	2.33
19° = rate of 4	14° = rate of 3	2.66
21° = rate of 6.5	14° = rate of 4	2.32

An examination of the data in the foregoing tables shows that the gastric hunger contractions of the frog within certain not very wide ranges of temperature follow the rule of van't Hoff and Arrhenius, and furthermore, that the relation between the contractions and the temperature is maintained either when the animal passes from a lower to a higher temperature or vice versa. Hence, it may be concluded that the gastric hunger contractions of the neuro-muscular apparatus is essentially dependent on chemical processes which are evolved within the stomach muscle itself. In table 2, where the temperature was diminished there appears to be an increase in the value of the coefficient as zero is approached which is entirely in harmony with the observations of Riddle (46) and Rogers (47). The observations in tables 1 and 2 were made during the month of August, 1915, and three separate animals were used for the results in each table and the findings herewith set forth were verified by control experiments.

I have also been able to carry through a sufficient number of experiments to determine the exact points at which high and low temperatures absolutely inhibit the gastric hunger contractions. The temperatures at which the hunger contractions are completely inhibited are practically constant, for both high and low temperatures and do not vary more than 0.5° in either direction in all the experiments. The average temperatures at which these contractions are completely inhibited is 35°C. for the high temperature and 13°C. for the low temperature. Lowering the temperature 0.5° or at the most 1° in the first case, or raising the temperature 0.5° or at the most 1° in the latter case starts the gastric hunger contractions again. These experiments were also performed during the month of August, 1915. The intensity of the contractions have a maximum between 15°C. and 32°C. and a minimum at lower or higher temperatures, that is, up to that limit where gastric standstill is produced. Raising the temperature also increases the strength of the contractions as well as the rate, but this effect disappears sooner than the effect of increased rate.

It is a well-known fact, that amphibians may be cooled down to −1°C. (50) and that their limbs may be frozen in ice (51), yet if the heart is not frozen these animals survive, but just how does this freezing temperature affect the gastric mechanism? Two of the animals were subjected to temperatures of 7°C. by means of the freezing mixture already described. The hunger contractions in a short time were completely inhibited and the limbs of the animals froze and became stiff and hard and when removed later from the cooling chamber and placed on the table, they rattled like stones, although their bodies were not completely frozen. Both the animals appeared lifeless except for a very slow heart beat and infrequent respirations and their eyes were closed as if asleep. One hour later at room temperature these same animals were active and began to show feeble gastric contractions which increased in intensity during the next twelve hours but never reached the normal. These observations indicate that the gastric mechanism is capable of movement at all seasons of the year and even in hibernation when given the suitable temperature. If the temperature change is gradual, as at the rate of 10° per three hours the animal is not disturbed, but passes into a drowsy or sleepy state.

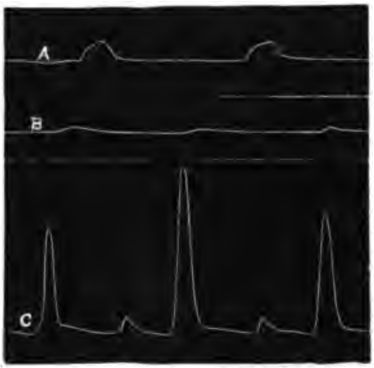

Fig. 7. Gastric hunger contractions of the turtle at different seasons of the year. *A*, contractions on a moderately cold day in winter (January). *B*, contractions on a very cold day in winter (January). *C*, contractions two and one-half days after the break of winter (March 28), weather mild and warm.

The seasonal variations as artificially produced on the frog have been verified on the turtle during the past year. Records of the gastric movements have been taken through the changes in the seasons from August, 1915, to May, 1916, inclusive. In all instances the activity of the gastric motor mechanism followed the climatic changes in temperature (fig. 7). In the coldest days of winter when the turtles showed no gastric activity or in only minor degrees, placing the animals in a tank of warm water for half an hour would greatly augment the gastric movements. However, the gastric hunger movements in winter are never as vigorous as in summer as is shown by my series of experiments. During the winter the turtles took no food although it was offered to them many times.

X-RAY STUDIES ON THE FROG'S STOMACH AND OBSERVATIONS ON THE EXCISED STOMACH

The general characteristics of the stomach movements in the frog have been discussed. We have now to consider the genesis of these movements. Two methods of investigation were used, namely, the X-ray and the excised stomach. The X-ray method consisted of introducing into the stomach a double walled balloon, the walls of which were separated by a thin layer of bismuth subnitrate mixed with vaseline to form a thin paste (52). The balloon was then connected with the usual recording apparatus and the fluctuations of the float in the manometer served as an indicator for studying the different phases of the gastric movements with the X-ray. From the X-ray studies made on the frog's stomach by means of this bismuth coated balloon, the gastric hunger contractions were found to be peristaltic, the peristaltic waves originating within about 1 cm. of the cardia and then advancing rhythmically over the entire stomach toward the pyloric end increasing in strength as they proceeded. These observations on the peristaltic activity of the stomach are in agreement with the X-ray findings reported by Cannon (53) in the dog, cat, rabbit, guinea-pig and rat, and by Roux and Balthazard (54) in man, dog and frog. For the X-ray observations the frogs were placed in the box already described for the cooling experiments and this was then placed over the tube generating the rays which was properly protected. Thus by looking through a fluoroscope the gastric hunger peristalsis could be watched with the X-rays. A series of X-ray photographs was taken of the balloon in the frog's stomach during the periods of rest and at varying intervals during the period of gastric hunger contraction, the special radiographic plate being inclosed in the red envelope and carefully placed on the frog's back (figs. 8 and 9). In every case the contraction and the relaxation of the stomach corresponded precisely with the graphic record (fig. 1).

The studies on the excised stomach showed the same general phenomena as presented by the X-ray observations above. The stomach was removed with the esophagus and the intestine. The balloon was introduced into the stomach and the esophagus ligatured to the tube upon which the balloon was attached. This was connected with the recording apparatus and the stomach was placed in Ringer's solution at 30.5° C. which was found to be a most favorable temperature for gastric activity from the temperature experiments and into this solution was led a slow current of oxygen. The rate of the contractions increased but the amplitude was lessened as compared to the normal gastric movements (fig. 10). In other words, the stomach had lost its regulatory apparatus, the extrinsic nerves which govern normally the rate, size and strength of the gastric movements. These contractions from the excised stomach showed an average duration of about thirty seconds with practically no intervals of rest between the individual contractions. Increasing the pressure in the balloon does not necessarily raise the tonus, the stomach simply dilates until the limit of expansion is reached. This confirms the results on the living animal obtained from the stomach in situ which have already been described. Dropping the temperature even a few degrees reduces the size of the

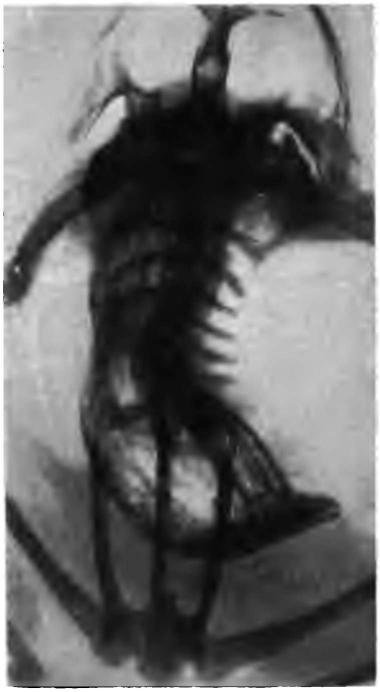

Fig. 8

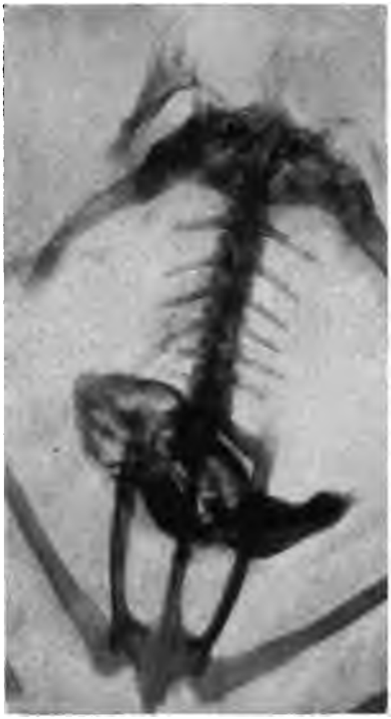

Fig. 9

Fig. 8. X-ray photograph of the bismuth balloon in the frog's stomach when the graphic record shows the stomach to be quiescent.

Fig. 9. X-ray photograph of the bismuth balloon in the frog's stomach at the height of a very strong hunger contraction.

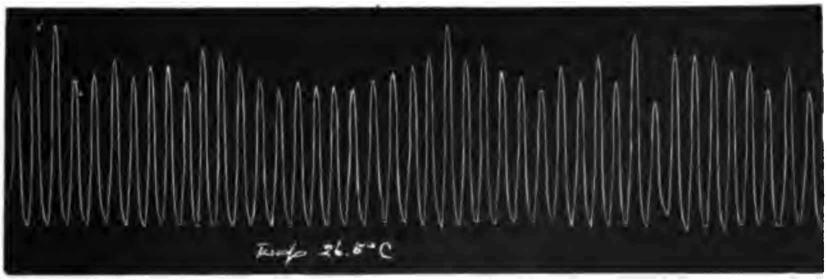

Fig. 10. Tracing from the excised stomach of the frog showing a marked tonus rhythm. Water manometer.

contractions. The curves show a distinct tonus rhythm with practically no changes in the general gastric tonus. The stomach also shows contractions with an uninflated balloon in it, as well as when it is completely empty and collapsed, but the peristaltic contractions are feeble, due to the fact that the organ is collapsed and not in a suitable condition to exhibit the full strength of the contractions. The cardia, however, is the first part of the stomach to exhibit motor activity and the last part to show it. The peristaltic waves appear to originate at the upper end of the gastric tube at a tonus ring near the cardia which forms a more or less marked depression known by X-ray workers as the "incisura cardiaca" (55), and from this point the waves sweep over the stomach toward the pyloric end as in the case described under the X-ray observations. The peristalsis observed in the frog's stomach does not always bear the character of rapidly running waves, but in certain cases may have a tonic character similar to the tonic contractions forming deep rings, thus giving the stomach the form of an hourglass but when these contractions are carefully analyzed, they are found to be peristaltic throughout. These observations are in confirmation with those of Pletneff (56) on the isolated stomachs of cats and pups with the exception that the peristaltic waves begin at the middle of the stomach rather than near the cardia, but they are contradictory to the findings of Dixon (22) and Morishima and Fujitani (29) who claim that the contractions of the stomach wall follow no strict conformable order and that the contraction rings generally show little tendency to spread, therefore according to these authors the movements of the frog's stomach are not peristaltic. However, X-ray observations by various investigators have conclusively shown the movements of the stomach to be peristaltic.

THE EFFECT OF DECEREBRATION UPON THE GASTRIC HUNGER MOVEMENTS OF THE FROG

We know, particularly through the researches of Carlson (57) on the higher animals (man and dog) that the gastric hunger contractions are inhibited by psychic stimuli and that at least in the dog they continue even after the stomach has been isolated from the central nervous system by section of the vagi and splanchnic nerves. Under these conditions stimulation of the gastric mucosa also produces the characteristic reflex inhibition but to a lesser degree than normally. It is also known that the stomach passes into a permanently hypotonic condition after double vagotomy (33). King (40) working with decerebrate guinea-pigs has reported that the rate of the gastric contractions is increased although similar in character to those from the normal animal and also that the stomach becomes hypertonic. However, in the frog the gastric hunger contractions are not affected by the removal of the cerebral hemispheres, the graphic record of the normal and the decerebrate animal remaining practically the same (fig. 11). Since the removal of this organ produces no apparent effect on gastric motility it may be inferred that what controlling influence the brain exercises over the stomach of this animal comes from centers in the mid-brain and medulla and not from those of the cerebrum. This idea is in agreement with the more recent work of Miller (58) on dogs, who by means of unipolar stimulation in the floor of the fourth ventricle has localized in the dorsal vagus nucleus (ala cinerea) centers for cardiac inhibition and for movements of the stomach and small intestine. In the higher animals cerebral processes of pleasantness such as the appearance of a friend and unpleasantness such as fear, anger, fright, etc., reflexly inhibit the rate and tonus of the gastric movements, whereas in the case of the frog similar reflex inhibitory action on gastric motility is only exhibited to a very slight degree. This demonstrates again the simplified gastric mechanism of the frog and that the cerebral processes exert no appreciable influence on the gastro-neuro-muscular apparatus. Eight animals were decerebrated all of which confirmed the above results, even one in which the semi-circular canals were injured and all exhibited the characteristic exaggeration of the reflexes. In (fig. 11, *B*) the contractions do not appear quite as high as in the

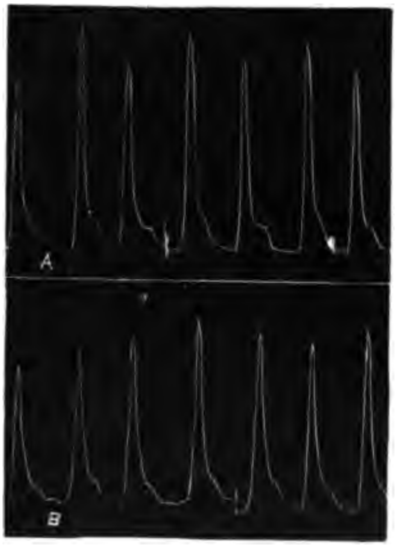

Fig. 11. Records from the stomach of the normal and decerebrate frog. *A*, contractions from the normal stomach after three and one-half days' fast, stomach empty. *B*, contractions from the same animal's stomach twenty-one hours after decerebration. Note that the contractions in *A* and *B* are practically identical in rate and form. Water manometer.

normal animal, however, in other cases they have been found to even exceed the normal.

SUMMARY

1. The simpler gastric mechanism of the frog does not show the distinction between the digestive peristalses and the hunger contractions present in the higher animals. There is no increase in gastric tonus and the hunger contractions are practically continuous.
2. The gastric hunger movements of the turtle show a periodicity, a feature present in the higher animals. There are slight increases in gastric tonus and two types of gastric activity are exhibited.
3. The hunger contractions in both the frog and turtle are inhibited or weakened by the introduction into the stomach of small quantities of water, weak alkali and acid, but the inhibitory effects are not produced as quickly as in the higher animals. When these substances are introduced into the mouth of the frog the inhibitory effects are very slight.
4. Water, weak alkali and acid when introduced into the filled stomach of the frog inhibit the digestive peristalsis in the same degree as they inhibit the hunger contractions of the empty stomach. In the higher animals normal digestive peristalsis is not inhibited by these substances in the same amounts.
5. During prolonged fasting in both animals the hunger movements are greatly augmented and the contractions show a marked increase in the amplitude directly proportional to the length of the fast, but no increase in gastric tonus. In the higher animals like the dog there is a marked increase in the gastric tonus in prolonged fasting, and this increase is inversely proportional to the decrease in the amplitudes of the hunger contractions.
6. Within certain not very wide ranges of temperature the hunger movements of the frog's stomach fall within the limits of van't Hoff's rule.
7. The hunger contractions of the frog's stomach are completely inhibited at 13°C. for low temperature and at 35°C. for high temperature. Raising the first temperature or lowering the second 0.5° or at the most 1° starts the hunger contractions again.
8. In both animals the gastric hunger activity is proportional to the climatic changes in temperature within certain limits as is indicated by van't Hoff's rule.

9. The gastric hunger movements are peristaltic and the waves start near the cardia at the "incisura cardiaca."

10. The stomach of the decerebrate animal behaves in the same manner as that of the normal frog.

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THE PHYSIOLOGY OF THE ATRIO-VENTRICULAR CONNECTION IN THE TURTLE

I. DISTURBANCES OF A-V CONDUCTION

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INTRODUCTION

In a former paper (1) a functional differentiation in the A-V funnel of the lizard and turtle was demonstrated, in that the right and left parts of this funnel when reduced to a very narrow bridge could, either of them, conduct the contracting impulse from the A to the V, so that there was coördination between them. Furthermore it was found that if one of the parts of the connection were alone cut, there resulted a severe and long lasting block, while if both were cut permanent dissociation between A and V followed. In two later papers (2 and 3) the anatomical grounds for this physiological differentiation were shown to exist in a more intimate connection between the right and left funnel fibers and those of the inner wall of the ventricle than at other parts of the funnel.

After the publication of my first paper two others dealing with this same general subject appeared. Nakano (4) showed that the dorsal, right and left parts of the A-V funnel of the frog and salamander possessed a higher degree of conductivity than the remaining parts. And Mangold and Kato (5), from an analysis of the effects on the electrocardiogram of cutting and piercing the A-V connection in the fowl, have traced the pathways of the contracting impulse, which begin in the "sinus node" (6) and are collected at the level of the A-V boundary into a bundle, the principal branch of which goes into the right ventricle along the septum, while the other goes through the septum to the left and connects with the papillary muscles. Mangold (7) has given a summary of all these results.

To carry on the investigation of this problem of functional differentiation in the A-V connection a physiological and anatomical study of this region in the alligator's heart has been undertaken, the results of which will be published shortly.

Wishing to continue the investigation of this differentiation in the turtle heart it was necessary to repeat the earlier experiments that had been carried out on the heart of the lizard and of the turtle (Clemmys lutaria) on a native species. Malacoclemmys geographica was the species used.

EXPERIMENTAL

The methods of experimentation can be briefly described. The cervical cord was cut through and the brain and spinal cord destroyed. The plastron was then removed as rapidly and with as little loss of blood as possible and the heart laid free by slitting up the pericardium. Before and after opening the pericardium the frequency of beat was taken with a stop-watch. The heart was then suspended from the right auricle and the apex of the ventricle. In some cases the heart was excised, in others left in situ and a record of the "normal" heart taken for frequency of beat and length of A-V interval. To determine the parts of the funnel of greatest conductivity portions of this were cut through with a pair of fine scissors, one of two procedures in the manner of cutting being followed. Either a series of small cuts was made and after each a record taken to observe the effect of the cut on A-V conduction, and the cutting thus continued until an interference with the coördination of A and V took place, which was then allowed the opportunity of recovering, after which the process was repeated. Or a large portion of the funnel down to a desired narrow bridge of tissue was cut through at once, and a record taken to observe the resultant degree of block and its recovery.

It must at first be pointed out with regard to the study of the A-V conduction time, etc. that, as has been earlier noticed (1 and 4), every mechanical disturbance of the heart has some effect on its activity, which may express itself in a retardation or acceleration, or a short standstill of both A and V or of V alone, extra systoles, group formation, pulsus alternans, ventricular fibrillation, etc., all of which are of short duration and are not to be regarded as typical effects of the particular operation. Lewis (8) noted that once the pericardium is opened irregularities in the beat occur which he considers due to changes in temperature and in the position of the animal. Meek and Eyster(9) also note that handling the heart leads to irregularities.

RESULTS

Briefly stated, the results obtained by cutting through parts of the funnel of Malacoclemmys substantiate in the main those obtained with Clemmys. Following the plan of Nakano (4) attempts were made to divide the ring into eight parts, but it was soon found that by this nothing was to be gained. Particular attention was also paid to the dorsal portion of the connection, since Nakano had found that in the frog this was an important part, but with negative results.

The right and left parts of the funnel were again found to be possessed of a higher degree of conductivity than the other parts. But my former results with regard to the consequent marked and long lasting block when the one or the other of these parts was cut through, and the permanent dissociation produced when both were severed have not been obtained. For in Malacoclemmys the broad ventral and dorsal portions of the funnel remaining when the right and left sides are cut through are fully capable of conducting the contracting impulse so that the ventricle, after longer or shorter standstill, beats again, either in a low degree of partial block which shortly recovers, or immediately coördinated in a 1 : 1 rhythm. In other words no part of the connection in Malacoclemmys can be said to be indispensable for the coördinated conduction of the impulse to the V. But nevertheless, as in the heart of Clemmys, the right and left parts of the funnel are possessed of a higher degree of conductivity. For along either of them when all other parts are severed, and when they are reduced to a mere thread of tissue incapable of being further narrowed, the impulse from the A is able to reach the V and cause it to beat coördinated with it and in a normal 1 : 1 rhythm.

The cutting of any part of the connection increases the length of the A-V interval to a greater or less extent, but with apparently no reference to the portion severed (see table 1). In contrast to the relatively slight effects on lengthening the A-V interval when a particular portion or portions of the funnel are severed are those when all but a certain small part is cut through. Looking over these examples it will be apparent that in the heart of Malacoclemmys, as in Clemmys, the right and left parts of the funnel are the most functional, not only from the standpoint of it being possible to narrow them down more than the dorsal and ventral portions without affecting coördinated conduction or obliterating it entirely, but also from the standpoint that when narrowed down to so small a bridge of tissue that another cut

TABLE 1

Effects of cutting parts of the A-V connection on the length of the A-V interval

NO.	TEMPERATURE	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	RETARDATION	FREQUENCY	REMARKS
7	19.5	10.35		1.12		18	
		10.38	Right and left, leaving broad ventral and dorsal connection	1.20	0.08	17	Short standstill of V, followed by 1 : 1
8	18.2	9.20		0.97		18	
		9.26	Right and left deeply	1.41	0.44	18	Short standstill of V, followed by 2 : 1, quickly 1 : 1
		9.28		1.31	0.34	17	
10	20.0	10.56		0.72		22	
		10.59	Right and left deeply	1.62	0.90	20	Short standstill of V, followed by 1 : 1
11	22.8	8.57		0.83		24	
		9.00	Right and left lightly	0.95	0.12	24	
		9.02	Right and left deeply	1.03	0.20	24	Short standstill of V, followed by 1 : 1
2	22.0	9.45		0.96		18	
		10.00	Right	0.96	0.00	18	
3	22.0	9.25		0.89		18	
		9.28	Left	0.94	0.05	17	
24	22.0	10.00		0.86		18	
		10.06	Left	0.94	0.08	18	
25	21.0	9.35		0.79		19	
		9.40	Dorsal	0.87	0.08	18	
5	21.2	10.24		1.04		18	
		10.28		1.08		16	
		10.30	All except left				Partial block, recovery to 1 : 1
		10.50		1.62	0.54	17	
3	22.0	9.20		0.88		18	
		9.25		0.89		17	
		9.28	Left	0.94	0.05	17	
		9.30	All except narrow bridge on right	1.62	0.68	17	Short partial block, followed by 1 : 1

TABLE 1—Continued

NO.	TEMPERATURE	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	RETARDATION	FREQUENCY	REMARKS
6	18.3	9.20		0.95		21	
		9.23 to 9.44	A series of cuts leaving bridge on dorsal	1.88	0.93	20	
		9.45		1.93		20	At 9.46 irregular dropping of V systoles, ending in dissociation
12	20.9	9.22		0.99		24	
		9:26	Right and left lightly	1.08	0.09	24	
		9.28 to 9.33	All remaining except dorsal	1.60	0.72	24	Fibrillation of V, followed by 1 : 1
7	19.5	10.35		1.12		18	
		10.38	Right and left lightly	1.20	0.08	17	
		10.42	Dorsal, leaving ventral			18	Standstill of V for three minutes, then 4 : 1, 2 : 1 and at 10.46 1 : 1
		10.46		1.32	0.20	18	
		10.50	Ventral bridge narrowed			18	Permanent block

would necessarily sever it the A-V interval is not lengthened so much on the average as when the dorsal or ventral portion of the funnel alone remains.

It is clear that whether and to what degree a retardation of the A-V interval will take place depends not merely upon general injury, such as the length of the experiment and the number and extent of the cuts made, but also upon the importance for A-V impulse conduction of the part injured. The width of the bridge of tissue is less important for coördinated conduction than the part that has been left. There is an independence also between the ability to conduct and the velocity of conduction. For whenever a partial block has been produced which later disappears the A-V interval is longer than it was before the block occurred.

Gaskell (10) from a few experiments, which were far from complete or conclusive, on cutting away various parts of the A-V connection, stated that the right ventral portion of the connection just under the aorta ("the upper portion of the right ring") was the most important part in the conduction of the contracting impulse from A to V. (Although there is no connection here between the musculature of the A and V, since the funnel musculature goes over into that of the bulbus (2 and 3)). Meek and Eyster (9) have found that the impulse in going from the A to the V reaches the various parts of the ring in the following order: anterior part, left part, right part. They call attention to the similarity between their results and those of Gaskell, and also believe that they have substantiated the old belief that the contracting impulse is of the nature of a wave which sweeps over the sinus and auricles and spreads downward from the circular fibers of the A-V ring over the V to terminate at the apex. Negativity in the basal portions coming first is supposed to be largely determined by proximity to the ring, although they do not exclude the possibility of there being certain pathways along which the wave may pass more directly or with greater ease, as shown by their results on the earlier negativity of the left part.

Somewhat opposed to these results are those of Lewis (8) obtained with the heart of Testudo graeca. According to him the distribution of the excitation process over the ventral surface of the V is that, as a rule, the base and central region, or the central, is earliest and the apex the latest. Sometimes he obtained a high reading from the region of the base in the neighborhood of the truncus arteriosus and sometimes the right margin was found to be activated at a later time than the left. The lateral and posterior surfaces of the heart showed the same distribution. From a comparison of readings taken from the inner surface of the V and compared with those from the outer, Lewis comes to the conclusion that there is in the V no special tissue which conducts more rapidly than the surface muscle fiber, as he found also to be the case in the heart of the toad. It is interesting to note that he obtained, table 4, p. 233, in the toad the earliest internal reading near the ring in front at the left auricular border, and that on p. 243 he makes the statement that the earliest internal readings are taken from the portion of the lining which borders the A-V ring, though he does not state just what part of the ring is the earliest. It remains to consider Lewis' idea of the manner in which the impulse from the auricles is communicated to the V (see his fig. 6, p. 236). He regards it not as in the nature of a simple surface spread, but, as in the V of the dog, a distribution from within outwards from the ring to the surface through the wall of the heart. "The musculature of the A and V do not meet at the surface of the A-V line but the ring is prolonged as a tube for some distance into the V; passing downwards it meets and fuses with the processes of muscle which radiate towards the central surface zone and apex of the heart. The trabeculae first carry the excitation to the central surface zone of the heart. Later the wave courses farther toward the apex and at the same time mounts to the base."

The results of these investigators are given to point out the want of agreement which exists between them, and how they both differ from what has been found by the method of severing portions of the A-V funnel. Perhaps a word more may be added regarding the arrangement of the fibers of the funnel. In the first place the physiological differentiation in the right and left portions of the funnel has been explained, (2 and 3), by the closer connection here. On the other hand the ventral and dorsal parts of the funnel are the first to come into union with the fibers of the ventricle and we might therefore expect these portions of the ventricle to become negative earlier. But Lewis finds that the same thing occurs on the lateral surface of the ventricle, so that it may be said that the places of earliest negativity are where the funnel musculature fuses with that of the ventricle. From Lewis' diagram and tables it can be seen that the heart of the tortoise differs from that of the toad in that the excitation wave reaches the basal portion of the surface of the heart of the tortoise before it reaches the apex, while the surface basal muscle of the toad heart is the last to be activated. This is in all probability due to the reason which Lewis (p. 246) gives for this chief difference between the distribution of the excitation process in the toad's heart and in the tortoise heart, namely, that in the tortoise ventricle the fusion of the ring musculature with the ventricular wall takes place at a level relatively nearer to the base than in the toad's heart.

It would be completing a detail of this phase of the problem of A-V conduction to study tracings of the passage of the wave of negativity before and after cuts of the kind above described were made. This would undoubtedly bring out more clearly the effects of the severing of this or that part of the funnel and the effects on the velocity of conduction between A and V.

DISTURBANCES OF A-V CONDUCTION

The disturbances of A-V conduction which are brought about by means of cutting the connection are of considerable general interest. As earlier stated with every cut made through a part of the A-V funnel there is, as a rule, an increase in the length of the A-V interval, without necessarily producing a condition of block (see table 2). This increase either becomes spontaneously greater or remains constant, and even in a few cases with the improvement in conduction decreases.

LENGTHENING OF A-V INTERVAL LEADING TO PARTIAL BLOCK

Of much greater interest than the mere slowing of conduction between A and V are those cases in which the retardation leads to the production of block (partial). The various degrees of such disturbances can be observed with great clearness in the turtle heart. A short description accompanied by curves and tables will now be given of them.

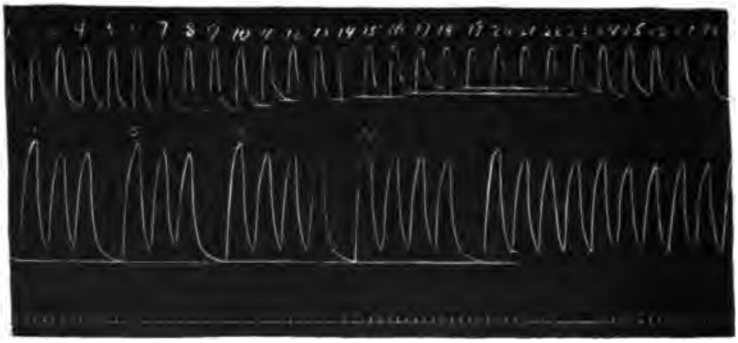

Fig. 1. Partial block characterized by gradual increase in length of A-V interval.

As is well known one of the types of partial block is characterized by a gradual increase, with every heart cycle, of the A-V interval until a contraction of the V is missed (11 and 12). During the pause the V, or the connecting system, recovers and the next cycle has a shorter A-V interval, which with every successive cycle again increases until another V systole is missed and thus the process is repeated. In figure 1 an example of such a type of block is shown and its final recovery to a normal 1 : 1 rhythm. The lengths of the A-V intervals are given in table 3 under No. 18 a. It will be observed that at the end where

TABLE 2

Effects of continued cutting of the A-V connection on the length of the A-V interval

NO.	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	FREQUENCY	REMARKS
3	9.20		0.88	18	
	9.25		0.89	17	
	9.28	Dorsal ligament and small cut on left......	0.94	17	
	9.30	All except a bridge on right................	1.62	17	
	9.40		1.60	16	
	9.50	Right bridge narrowed	1.71	16	Rhythm = 2 : 1
	9.53		2.09	16	Rhythm = 1 : 1
	9.58	Right bridge narrowed		16	Standstill of V for 2 minutes, followed by 3 : 1, recovery to 2 : 1
	10.26		1.77	15	Rhythm = 2 : 1
	11.20		1.79	12	Rhythm = 1 : 1
6	9.20		0.95	21	
	9.23	Right..................	1.06	22	
	9.25	Left...................	1.31	21	
	9.29	Right and left more deeply on dorsal.....	1.51	20	
	9.35	Ventral................	1.74	20	
	9.41	Septum................	1.76	20	
	9.44	Dorsal slightly narrowed...............	1.88	20	
	9.45		1.93	20	
	9.46			20	Block, and finally dissociation
8	9.20		0.97	18	
	9.26	Right and left deeply..	1.41	18	Short standstill of V, 2:1 rhythm of short duration, 1 : 1
	9.28		1.31	17	
	9.30	Ventral entirely through..............	1.71	16	
	9.34		1.60	16	
	9.38	Dorsal slightly from right.................	1.71	16	
	9.44	Dorsal slightly from right................	1.62	15	Rhythm = 2 : 1
	9.45		1.91	15	Rhythm = 1 : 1
	9.50		1.71	15	
	10.09		1.77	15	
	10.11	Dorsal narrowed from left..................		15	Standstill of V, later an independent V rhythm

TABLE 2—Continued

NO.	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	FREQUENCY	REMARKS
10	10.56		0.72	22	
	10.59	Right and left deeply	1.62	20	Short standstill of V, followed by 1 : 1
	11.03	Dorsal................	1.39	21	Rhythm = 4: 1
	11.06		1.39	22	Rhythm = 2: 1
	11.07		1.85	22	Rhythm = 1: 1
	11.15		1.59	22	Rhythm = 1: 1
	11.18	Ventral narrowed......		22	V is still
11	8.57		0.83	24	
	9.00	Right and left lightly	0.95	24	
	9.02	Right and left deeper	1.03	24	Short standstill of V, followed by 1: 1
	9.04	Dorsal and ventral from left............		24	Dissociation
	9.07		1.48	24	Rhythm = 1: 1
12	9.22		0.99	24	
	9.26	Right and left lightly..	1.08	24	
	9.28	Right and left deeper..	1.36	24	
	9.32	Ventral................		24	Fibrillation of V, followed by 1 : 1 after a single large V_s
	9.33		1.60	24	

the block disappears the A-V interval has a length almost equal to that after which the V systole was in the habit of falling out. In all figures the upper tracing is of the contractions of the right auricle, the middle those of the ventricle. The bottom line is time in seconds.

Figure 2 illustrates the influence of A rate and irregularities in A beat in the formation of this type of block. A block between A and V had been caused at 9.54 by cutting through a part of the funnel. The remaining funnel fibers gradually recovered from the injury, the V beginning by giving single, isolated contractions at 9.55, and at 9.57 suddenly beginning to beat in a 4 : 1 rhythm. At 9.59 this became 2 : 1 and continued regularly so until 10.04 at which time the record shown was taken. The A and V are for the most part beating in a 2 : 1 ratio, but occasionally, for several successive contractions, the rhythm is 1 : 1. When the lengths of the A-V intervals are measured (see table 4) under 10.04 it is seen that they are of a constant value, 0.86 seconds (0.8 second has been added since the A lever is that much

TABLE 3

Lengths of A-V interval in cases of partial block. Note the increase in length of interval when the rhythm becomes 1 : 1

NO. 18 A		NO. 10		NO. 11		NO. 20		NO. 18 B		NO. 4		NO. 14	
A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V
1	1.68	1	1.31	1	1.39	1	1.46	1	1.79	1	1.45	1	0.99
2	1.84	2		2		2		2	2.02	2		2	
3	2.01	3	1.31	3	1.39	3	1.46	3		3	1.45	3	0.99
4		4		4		4		4	1.79	4		4	
5	1.68	5	1.31	5	1.39	5	1.46	5	2.02	5	1.08	5	0.99
6	1.90	6		6		6		6		6		6	1.20
7	1.96	7	1.31	7	1.39	7	1.46	7	1.79	7	1.22	7	
8		8	1.85	8	1.46	8		8	2.02	8	1.43	8	0.99
9	1.62	9	1.62	9	1.51	9	1.46	9	2.02	9	1.51	9	1.04
10	1.79	10	1.40	10	1.40	10	1.86	10		10	1.62	10	1.12
11	1.96	11	1.40	11	1.40	11	1.84			11		11	1.22
12	2.12	12	1.40	12	1.39	12	1.80			12	1.36	12	1.28
13						13	1.77			13	1.43	13	1.28
14	1.68					14	1.77			14	1.48	14	
15	1.84					15	1.68			15	1.52	15	1.04
16	1.96					16	1.74			16	1.48	16	1.16
17	2.12					17	1.68			17	1.52	17	1.28
18						18	1.68					18	1.28
19	1.74					19	1.71					19	1.16
20	1.96					20	1.68					20	1.16
21	1.96					21	1.71					21	1.16
22	1.96					22	1.68						
23	1.96					23	1.71						
24	1.96					24	1.68						
						25	1.65						
						26	1.60						
						27	1.60						

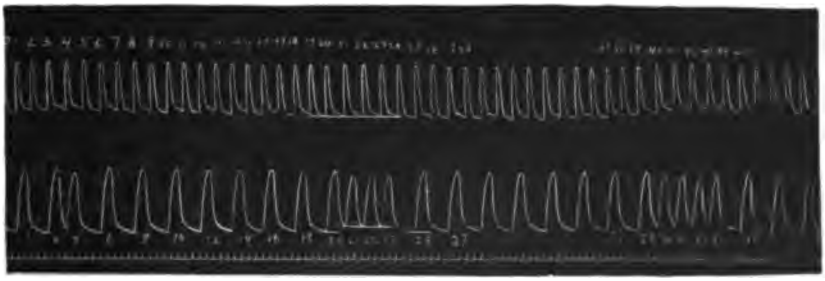

Fig. 2. Influence of A rate on partial block.

TABLE 4

Influence of A rate on formation of partial block (cf. figs. 2, 3 and 4)

10.04			10.10			10.22		
As	A-V	A-A	A_s	A-V	A-A	A_s	A-V	A-A
1	0.79		1	0.97		1	1.16	
		2.08			2.43			2.49
2			2	0.97		2	1.16	
		2.38			2.43			2.66
3	0.86		3	0.97		3	1.08	
		2.52			2.35			2.99
4	0.86		4			4	1.16	
		2.30			2.43			2.82
5			5	0.97		5	1.08	
		2.38			2.27			2.66
6	0.86		6			6	1.16	
		2.30			2.27			2.57
7			7	0.97		7	1.02	
		2.38			2.43			2.49
8	0.86		8			8	1.08	
		2.38			2.35			2.49
9			9	0.97		9	1.16	
		2.38			2.35			2.57
10	0.86		10			10	1.16	
		2.16			2.43			2.91
11			11	0.97		11	1.02	
		2.30			2.99			2.91
12	0.86		12	0.97		12	1.02	
		2.16			2.75			2.66
13			13	0.97		13	1.02	
		2.23			2.59			2.49
14	0.86		14	0.97		14	1.02	
		2.16			2.43			2.41
15			15	0.97		15	1.02	
		2.16			2.51			2.41
16	0.86		16	0.97		16	1.02	
		2.16			2.51			2.91
17			17	0.97		17	1.02	
					2.48			2.99
		2.23	18	0.97		18	1.02	
					2.43			2.74
18	0.86		19			19	1.02	
		2.16			2.43			2.49
19			20	0.90		20	1.02	
		2.16			2.43			2.49
20	0.86		21	0.97		21	1.02	
		2.66			2.51			2.46

TABLE 4—Continued

10.04			10.10			10.22		
A_s	A-V	A-A	A_s	A-V	A-A	A_s	A-V	A-A
21	0.86		22	0.95		22	1.02	
		2.66			2.43			2.41
22	0.86		23			23	1.02	
		2.52			2.43			2.99
23	0.86		24	0.90		24	1.02	
		2.38			2.51			2.66
24			25	0.95		25	1.02	
		2.30			2.43			
25	0.86		26					
		2.23			2.43			
26			27	0.90				
		2.30			3.08			
27	0.86		28	0.95				
		2.16			3.08			
28			29	0.95				
					2.59			
			30	0.95				
					2.67			
37	0.86		31	0.95				
		2.08			2.59			
38			32	0.95				
		2.16			2.59			
39	0.86		33	0.95				
		2.66						
40								
		2.52						
41	0.86							
		2.38						
42								
		2.38						
43								
		2.30						
44								

too far to the right). The A-A interval is, however, variable and shows a gradual decrease in length after A pauses which occur more or less regularly. These A pauses, or sudden lengthenings of the A-A interval allow the A-V connection (or the V) time to recover between the A systoles so that the impulse reaches the V. Perhaps too the strength of the A impulse is greatest after the pause and decreases gradually as the rate increases. After the pause the rate of beat increases as evidenced by the gradual shortening of the A-A interval until finally the A-V rhythm becomes 2 : 1, the A-V connection being able to conduct only every other impulse, or the V to respond to only every other.

In figure 3 a later record of the same heart is shown taken at 10.10 (see table 4). The block has improved so that the rhythm is for the most part 1 : 1. The A-V interval has increased somewhat being now 0.97 second and constant (0.7 second has been added). The rate of A has decreased, being now about 25 per minute. Here the same tendency on the part of the rate to gradually increase is manifest so that the rhythm either becomes 2 : 1 or a V systole is missed. Again the pause, lengthening the A-A interval, lets the impulse through every time and the rhythm becomes 1 : 1 to remain so until the rate of A becomes too rapid.

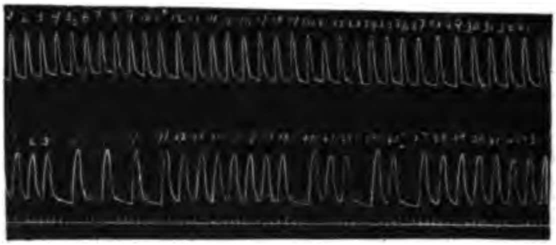

Fig. 3. Same as figure 2.

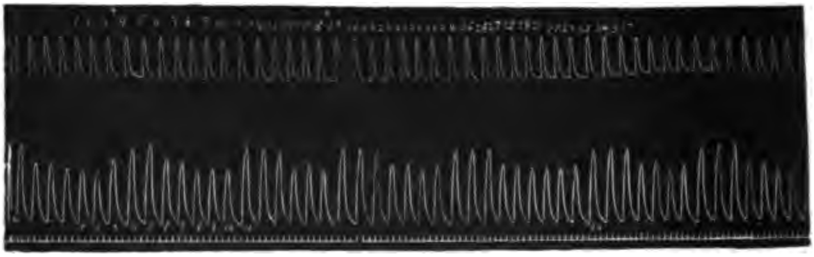

Fig. 4. A further influence of A rate on the character of V beat.

The influence of auricular pauses is still further shown in figure 4 which is a record of the same heart taken at 10.22 (table 4). The A-V interval (0.7 second added) is practically constant, showing only slight variations which, however, do not give evidence of having any influence on the V contractions. The frequency of A beat is now about

23. Every time the A pauses, to beat again with a lengthened A-A interval which gradually decreases, the following three V systoles are larger, after which the A rate becoming more rapid the strength of the impulse getting through the A-V connection is diminished and fewer fibers of the V are excited to contract and, therefore, the V systole is not so vigorous.

It is possible to obtain all grades of this type of partial block, though the most common cases are those in which every second, third or fourth V systole falls out. Many cases of the other type of partial block were also observed and tracings made showing the disappearance of the disturbance to conduction. Several points have been noted which seem of interest. In figure 5 is shown an example of the transition of a 4 : 1 to a 2 : 1 rhythm, the values of the A-V interval being 1.33 seconds before and after the transition (4.0 seconds subtracted). In figure 6 is shown the final transition to 1 : 1 (see table 3, No. 10, 3.6 to 4.0 seconds subtracted). Note that the A-V interval is increased at the time of transition. In the next column (No. 11) are given the values of a case where the A-V interval though increased at the time of transition quickly returns to its previous value. No. 20 represents another case with a marked increase in length of A-V interval as the block disappears. This at first decreases and later develops an alternation of a slightly longer and a shorter A-V interval, and a corresponding higher and lower V contraction or pulsus alternans. It is to be

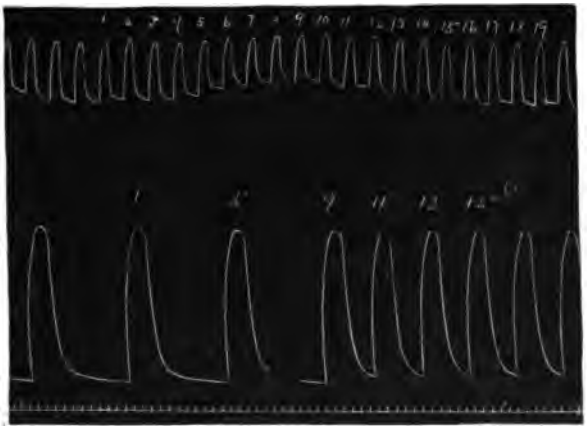

Fig. 5. Recovery of a 4 : 1 rhythm to a 2 : 1.

noted in all that at the moment of transition there is a greater or less increase in the length of the A-V interval, which may later disappear, or decrease more or less.

Sometimes the transition from a 4 : 1 rhythm takes place to a 3 : 1 (instead of to a 2 : 1) which eventually changes to a 2 : 1 rhythm. A characteristic of all cases which have been registered of a 3 : 1 rhythm changing to 2 : 1 consists in the apparent inability of the reduced (injured) A-V connection to allow the increased strength of impulse to get through once and for all. There is always a return for one or more cycles to a 3 : 1 ratio after which the 2 : 1 rhythm is permanently attained.

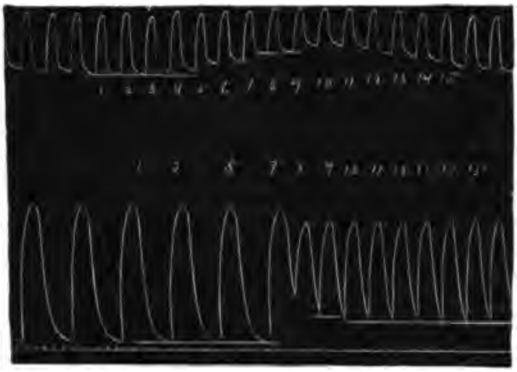

Fig. 6. Recovery of a 2 : 1 rhythm to a normal 1 : 1.

Figure 7 shows a very brief instance of this sort of thing. The V had for some time been contracting after every third A systole. Suddenly after A, 9, the V contracted thus missing but one A beat instead of two. After that it again missed two beats and then followed regularly after every second A systole. Sometimes it takes very much longer for the 2 : 1 rhythm to be attained and the alternation between it and the 3 : 1 rhythm lasts for some time.

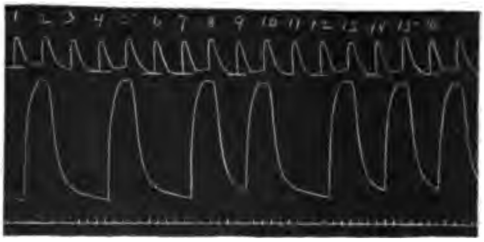

Fig. 7. Recovery of a 3 : 1 rhythm to a 2 : 1, showing the inability to make the change once and for all.

A block represented by a 2 : 1 rhythm may change, in addition to the method illustrated in figure 6, by passing through an intervening stage, characterized by the gradual increase in the length of the A-V interval (see fig. 8). At 9.37 the A and V were beating regularly in a 2 : 1 rhythm. Unfortunately the drum had to be stopped and before it was again started it was observed that not every second

V systole was being dropped, but every third, in other words the rhythm was now 3 : 2, as shown in the second part of figure 8. The values of the A-V intervals are given in table 3, under No. 18 b (0.4 second subtracted). This has become therefore a typical case of a gradual increase in length of A-V interval to a maximum after which a V systole is dropped. The records in these two figures are early stages

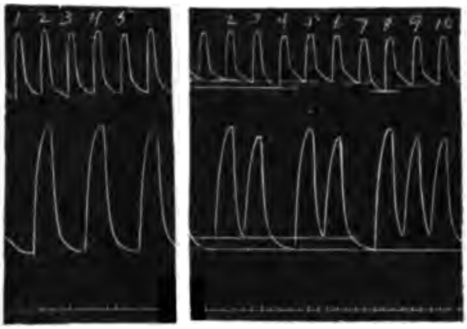

Fig. 8. A stage in the recovery of a 2 : 1 rhythm, showing intermediate conditions of 3 : 2 and 4 : 3 rhythm. Cf. Fig. 1.

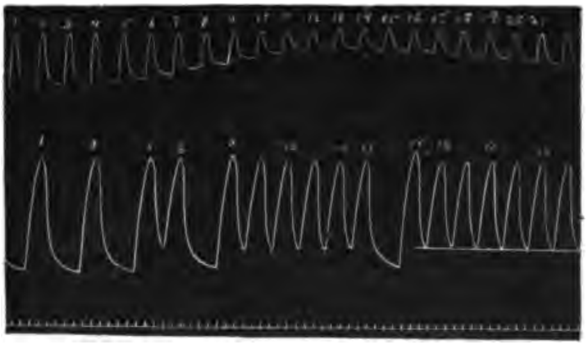

Fig. 9. Recovery of a 2 : 1 rhythm to a 1 : 1, passing through intermediate tages of gradually increasing length of A-V interval.

of the block illustrated in figure 1 (see table 3, 18 a). The ratio of A and V quickly became 4 : 3. At 9.40 (fig. 1) it is 5 : 4 and soon after this the block disappeared, and coördination took its place.

In No. 4 (table 3) the values of the A-V intervals of a much shorter case are shown, and in figure 9 another case is illustrated (see table 3, No. 14, 1.7 seconds subtracted). These cases are of particular interest in that they point to the view that these two types of block are closely related and that the type in which there is a gradual increase in the length of the A-V interval is intermediate between 2 : 1 block and coördinated rhythm, although a 2 : 1 block often disappears without passing through this intervening stage.

The results of these experiments are not entirely in accord with the view expressed by Gaskell to the effect that the degree of block is directly proportional to the thickness of the connecting bridge left between the A and the V, and that by gradually decreasing this, a higher degree of block is to be produced. As pointed out in the first portion of this paper, rather more emphasis is to be placed on the particular part of the connection left, since some parts of the funnel are more effective than others in conducting the contracting impulse. Partial block can of course be produced in the way in which Gaskell claims but only within limits. For if time is allowed it is nearly always seen that the remaining bridge is more functional than at first seemed to be the case, in that the degree of block decreases and finally disappears, the right and left parts of the connection having the greatest capacity for recovery.

VENTRICULAR RHYTHM

In Clemmys complete block was always to be obtained by severing the most important parts of the A-V funnel, namely, the right and left. In Malacoclemmys, as has been pointed out above, this is not the case. But complete block can very readily be obtained in this form by narrowing down the connection left after the right and left parts are cut through. This complete block may consist in the V becoming quiescent and remaining so as long as it was observed. Usually, however, the V after an interval begins to beat, at first slowly and gradually increasing in rate, but entirely independently of the A. The rate of the independent V rhythm has never been seen to approach that of the sinus rhythm, as was observed in the heart of Clemmys and figured in my earlier paper on pp. 199 ff., where in old hearts the V sometimes initiated the rhythm of the heart, and beat more rapidly than the A. (Some of these figures perhaps represent cases of very much lengthened A-V, rather than V-A). The rhythm producing power of the V, or rather of the A-V funnel, of Malacoclemmys does not seem therefore to be so highly developed as in Clemmys. But it can institute a rhythm as was shown to be the case in the lizard ((1) p.

168, fig. 33), where after the connection between A and V had been completely cut through, the V continued to beat and in the ratio of 10 contractions to 25 of the A. In one case of the development of a funnel rhythm a severe block was produced at 9.34 from which the connection gradually recovered letting through impulses so that the V gave isolated contractions which at 9.39 were at the rate of about 1 to 7 A systoles. At 10.10 and 10.20 the connection had much improved for the V was beating once to every three A beats and apparently coördinated. But a cut at 10.29 completely severing the bridge had no other effect than to slightly retard the V, thus showing the independent origin of the V rhythm. Figure 10 is a record of the heart beat after the A-V connection had been severed.

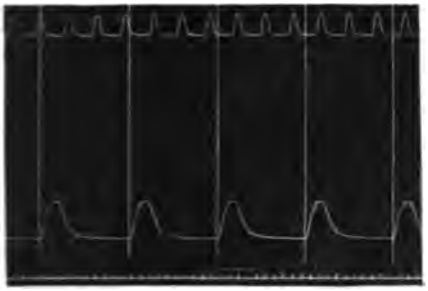

Fig. 10

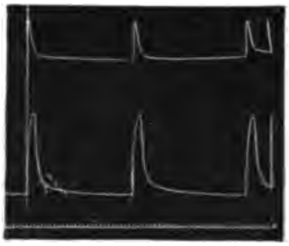

Fig. 11

Fig. 10. Independent A and V contractions after the A-V connection had been severed.

Fig. 11. An example of an A-V funnel rhythm.

To remove the possibility of this independent V rhythm being due to the Na in the Ringer solution with which the hearts were moistened a number of experiments were made in which the heart was placed in a chamber kept moist by filter paper saturated with tap-water. The results of these experiments are summarized in table 5. Several records were taken showing the rhythm originated in the A-V funnel after the sinus had been removed, and one of these is shown in figure 11. As well known, Engelmann (13) demonstrated a V-A rhythm in the frog after standstill following clamping off the sinus.

TABLE 5

Instances of the development of independent V rhythm

NO.	TEMPERATURE	FREQUENCY	TIME	OPERATION	RESULTS
52	20.5	22	2.15	First and second Stannius ligature	
		15	5.30		A is still, 1 V_s every 25–35 seconds
53	18.0	18	10.05	First and second Stannius ligature	
		17	12.20		A is still, 1 V_s every 40, 20, 35, 20, 35, 20, 35, 19, 24, 33 etc. seconds
54	18.4	21	10.30	First Stannius ligature	
		18	11.44		V and A beating about 3 per min.
			11.46		1 V-A cycle every 30, 27, 30, 35, 34, etc. seconds.
			12.45		1 V-A cycle every 30, 29, 29, 33 50, 22, 24, etc. seconds.
55	19.4	24	11.00	First Stannius ligature and V trimmed down so that only a small portion surrounding funnel remains	
		26	11.10		1 cycle every 10, 40, 27, 20, 23, 15, 27 etc. seconds.

DISCUSSION

A functional differentiation exists in the A-V funnel of Malacoclemmys geographica as in Clemmys lutaria. The right and left parts of the funnel possess in a higher degree than other parts the ability, when the one or the other is the sole connection between A and V and when reduced to a very narrow bridge, of conducting the A impulse so that A-V rhythm is coördinated. The production of severe and long lasting block when one or the other of these two parts is cut through, and the complete dissociation produced when both are cut through which was obtained with Clemmys and Lacerta have not been substantiated with this heart, and no part of the funnel can be said to be indispensable for coördinated conduction.

It will facilitate the discussion of the evidence regarding block given by the above results to briefly review the opinions of others concerning this phenomenon. Engelmann (14) explains the type of partial block, in which a V systole more or less regularly drops out after the gradually increasing A-V interval reaches a maximum, by the effect of the V systole on the length of the A-V interval. He assumes a summation of the retarding influence of the successive stimuli so that the A-V interval becomes larger and larger up to a maximum which is retained, or at which from greater fatigue a V systole is dropped. During the resting period the conductivity of the A-V connection is improved and the V contracts after the next A systole with a shorter interval or latent period, after the next A systole with a slightly longer interval and so on until a V systole is again dropped, and the process thus continued. Later (15) he concludes that the gradual increase of the latent period for excitation of the V plays a part in the falling out of V systoles which occur after the maximum value of the A-V interval has been reached, this increase in latent period pointing to a decrease in the velocity of conduction.

Muskens (16) who observed this type of block after vagal stimulation claims that it is due to blocking of the wave of excitation so that it does not reach the V, and in poorly nourished hearts, to the increased inability of the V to conduct after every V systole.

Hering (17) points out that there is no proof, excluding those cases in which the connecting fibers have been directly injured, that the dropping out of the V systoles is due to a temporary loss of conductivity on the part of the A-V connection fibers, but that the explanation is to be found in the loss of irritability of the V, in the lengthening of the refractory period (see also Straub, 18). He admits however that the gradual increase in the length of the A-V interval can indirectly cause the dropping out of a V systole by the lengthening of the refractory period of the V, but although he grants the possibility in certain cases of the failure of the A-V connection fibers to conduct causing the dropping out of a V systole, he sees nothing to induce him to accept the view. In a later publication (12) he refers the falling out of a V systole after a gradually increasing A-V interval to a retardation in A-V conduction which takes place in Tawara's node.

Von Kries (19) considers this type of block, occasional cases of which he obtained when the contractions of the V were slowed by cooling it, as due to the V systoles being shoved a little further and further apart until an auricular impulse falls into the refractory period of the preceding V systole, which results in a V systole being missed. After the rest the next V systole follows the A systole after a shorter interval and the process begins again. The impulses going from A to V must be assumed not to be momentarily concentrated but spread over a certain short period of time. Furthermore he points out the necessity of assuming in the heart muscle a refractory period for conduction after a stimulation as well as for contraction. It is interesting to note in von Kries' curve on p. 488 that the A systole corresponding to the V systole which is missed is lower than the other A systoles. Perhaps we have here an example of periodically weakened A impulses.

The other type of partial block is also thought by Engelmann (15) to be due to a retardation of conduction by which he explains rhythmic disturbances of the heart such as hemisystole and pulsus alternans. If a stimulus comes to the heart muscle at the moment when the ability to conduct has not returned to normal, then a partial contraction of the V musculature will result, and only later when all the fibers have regained their normal ability to conduct will the V as a whole take part in the systole. Differences in conductivity are not claimed to be always the explanation of such disturbances, and he admits that differences in contractility of the muscle fibers, temporarily weakened by contraction, are also important factors.

Erlanger (20) from experiments on compression of the bundle in the dog and on strips of turtle heart concludes that it is the strength of the impulse which determines whether or not it will, after having passed a partial block, serve as an efficient stimulus to the part beyond. He found it impossible to explain partial block by assuming an increase in the refractory period at the seat of injury. Furthermore, he accounts for the development of partial or complete block when the auricular rate is increased in part by the diminution in strength of the A impulses, and in part by the fact that more of the impulses fall into the ventricles before their irritability reaches the level at which the impulses of the normally beating auricles would have become efficient stimuli.

Hering (21) brings into line with his explanation of pulsus alternans this type of partial block. Pulsus alternans he considers due to the lengthening of the refractory phase of the V fibers which therefore do not respond to the stimulus. The periodic dropping out of a V systole is to be explained in the same manner, viz., by a lengthening of the refractory phase of conduction, so that the stimulus does not reach the fibers.

And finally von Kries (22), discussing block occasioned by narrowing the connecting bridge between A and V (p. 88), does not consider that merely by this procedure can conductivity be decreased, and that in order to explain the retardation in conduction it is necessary to assume a functional change in the remaining fibers of the bridge, a view tentatively stated by Gaskell (see Gaskell, p. 66). The fact that recovery from block takes place under these conditions makes this assumption highly plausible.

From this brief summary it is clear that considering the strength of the initial impulse as always the same there are two separate and distinct possible explanations of partial block. In the first the conductivity of the fibers of the A-V connection is assumed to be impaired on account of injury (actual cutting, compression or pathological conditions), so that there is a retardation of conduction or a failure to conduct on account of the lengthening of the refractory period. In the second the cause of block is sought in changes in the V, in a decrease in irritability or a lengthening of the refractory period, so that there is a failure to contract, or a failure to respond to the impulse brought to it from the A.

Both of these explanations are of course tenable, since it must be admitted that block is sometimes brought about by pathological changes in the A-V connection and sometimes by such changes in the V. Only those cases, however, in which a modification of the connection has taken place are to be considered here. It seems impossible to get away from the assumption that in such cases the resultant block is due to the injury which the fibers still present have suffered, and that some functional change takes place which retards the impulse or blocks it. Let us consider first the type of block in which the V contracts after every second, third, etc., A systole. The length of the A-V interval is increased. The strength of the impulse reaching the V is probably decreased by the cut, as Erlanger claims to be the case when the bundle is compressed, and the V therefore fails to be excited by every A impulse because the irritability of the V does not recover after every contraction rapidly enough for it to be excited. But how is the strength of the impulse decreased if not by the injury to the remaining fibers? And how is the increase in length of the A–V interval to be explained? According to Engelmann (14 and 23) we might say that the A-V interval increases because of the decreased strength of stimulus, or that the latent period of the V increases for the same reason. If the block remains stationary or even becomes greater this explanation might be sufficient. But when the degree of block becomes less or disappears, in other words when a stronger stimulus succeeds in getting through the connection, it is necessary to admit that the fibers of this connection have recovered in order to make this possible. Take a case of 4 : 1 block as it improves. When the reduction in the degree of block takes place directly to a 2 : 1, or to a 3 : 1 and finally to a 2 : 1 rhythm we note that the length of the A-V interval does not change or if it does it decreases slightly (see table 3). But when the block disappears and the A and V beat 1 : 1, it is almost invariably the case that the A-V interval is markedly increased at the time the transition is made. This increase in length of the A-V interval may quickly disappear or it may decrease to a greater or less extent but it usually remains considerably greater than when the beat was 2 : 1 (see table 3). The explanation of the improvement in A-V rhythm is that the conductivity of the connection fibers suddenly improves by recovery of the fibers from injury so that a stronger impulse gets through more often, or every time. Why the A-V interval is lengthened when the rhythm becomes 1 : 1 is possible of two explanations. A lengthening of the refractory period of the V so that it takes longer to contract or to be excited, or an increase in the refractory period of the fibers of the A-V connection. The second of these explanations is to me preferable. The impulse now getting through is presumably still weaker than it would have been if the cuts had not been made, and the fibers of the remaining bridge are still somewhat under the influence of injury. They cannot, therefore, conduct the impulse every time so rapidly, as they can every other time, the pause giving them an opportunity to recuperate their conductivity. The occurrence in many cases of a slight ventricular pulsus alternans just after the beat has become 1 : 1 is of interest in this connection. This again might be interpreted as evidence simply of a partial asystole, some of the fibers of the V not having returned to their full irritability, thus resulting in alternate V systoles being not so vigorous. But in many cases it is found that the A-V interval preceding the lower V systole is slightly shorter. This indicates that the A impulse gets through more quickly, and falls into the V at a time when some, or all of its fibers, have not regained their full irritability, and the following V systole is therefore lower. Next time the impulse, owing to fatigue of the connection fibers, takes longer to get through to the V. Its fibers are, therefore, more irritable and the following systole is larger. (No claim is made that all cases of pulsus alternans are to be explained in this way.) This condition of pulsus alternans continues until, owing to the increased improvement in the connection fibers, every impulse is able to get through at maximum speed and at maximum strength.

Now as to the other type of block in which the A-V interval gradually increases until a V systole drops out, after which the same thing is repeated. Here again the strength of impulse reaching the V is decreased, and the length of the A-V interval is increased in that the impulse travels more slowly due to the injury suffered by the fibers of the remaining connection. The general increase in length of the A-V interval from cycle to cycle is due to the gradually increased refractoriness of the connection fibers. Finally a V systole is dropped. This must be considered as due to the fact that the impulse does not get through and not that the impulse getting through is sub-minimal to the V, or that it falls into, or too near to, the lengthened refractory period of the V. After the pause the connection fibers recover their irritability and conductivity, and the first impulse gets through more quickly, the A-V interval is shorter. The V systole is of course more vigorous owing to the pause. The second impulse comes to the fibers of the bridge and finds them in a state of lowered excitability and conductivity owing to the passage of the previous impulse and this second impulse is weakened in turn and slowed, and the A-V interval is therefore still longer, and so on until the impulse is completely blocked and fails to get through.

In some cases the last V systole before a beat is missed is more vigorous than the preceding ones (see (1) fig. 49, p. 187). If the dropping out were due to the A impulse falling nearer and nearer into the lengthened refractory period of the V this could not possibly be the case; but if it is due to a slowing up and a weakening in the A-V connection, then the V would have a chance, waiting for the impulse to reach it, to recover in greater measure its irritability, so that the systole following is more vigorous. The failure of the next V systole to materialize is then plainly due to the failure of the impulse to get through to the V.

Quite often this type of block may be observed to be intermediate between a 2 : 1 rhythm and a normal rhythm, and either regressively or progressively. It has already been pointed out ((1), p. 195) that further cutting of the funnel converted a case of 3 : 2 block into a 2 : 1 block, and two cases are given above (see table 3, No. 4 and 14, and fig. 9) of the recovery of a 2 : 1 block, passing through a transitory stage of gradually increasing length of A-V interval to end with a dropping out of a V systole after which the beat becomes 1 : 1. Here again owing to recovery from injury on the part of the connection fibers the impulse able to get through the block suddenly became stronger but the connecting fibers for the time were not yet in a condition to take over every impulse without showing fatigue which they evidenced in a slowing up of the impulse and weakening it until it did not get through at all, thus causing a V systole to be missed after which a sufficient recuperation of the connection fibers took place so that the beat became normal. See also No. 18 (table 3, and figs. 8 and 1).

It has not been overlooked that a decrease in strength of stimulus alone may possibly explain both types of partial block, by the thereby relatively increased refractory state of the V, and that in the second type the gradually increased A-V interval may be explained by the gradually increasing length of refractory period of the V from cycle to cycle until an A impulse falls into it and the V cannot respond. Furthermore that the recovery from block may be simply due to an increase in the strength of the impulse getting through and the increased A-V interval to the increased length of the refractory state of the V on account of its being more often stimulated. This explanation may be the correct one, but at the present time the writer cannot rid himself of the conviction that injury to the A-V connection fibers is the ultimate cause of both of these types of block and that the failure of the V to contract is due to the failure of the impulse to reach it.

A word may be said regarding certain of my former experiments (1). In nearly all cases block was obtained by a method of gradual cutting which extended over a considerable period of time. This gave the irritability of the V time to become lowered and therefore warranted the assertion (p. 189) that the type of block characterized by a gradually increasing length of A-V interval was particularly often seen in dying hearts. Also those cases mentioned on p. 192 in which, although the A-V interval gradually increased, the V systoles did not fall out when the maximum length of A-V interval was attained, but followed the A systole after the minimal A-V interval, which gradually increased again and so on. All of these cases have been looked up and in all of them it has been found that they were truly dying, in that conditions were becoming worse instead of better, and therefore that the irritability of the V must have been decreasing. The fact noted on p. 194 that the V began to beat regularly with a length of A-V interval equal to that at which it usually fell out simply points to the recovery of the connection fibers and also to the view above expressed that the falling out of the V is not due to the A impulse coming to it when in its refractory period.

SUMMARY

1. There is a functional differentiation in the A-V funnel of Malacoclemmys as was found in Clemmys and in the lizards Lacerta viridis and agilis. When the auricles are partially separated from the ventricle, a connection on the right or left, though consisting of but a thread of tissue, in contradistinction to other parts of the funnel, can conduct the contracting impulse so that A-V coördination is preserved or restored.
2. Cutting the right or left parts of the funnel, however, does not necessarily produce block as was found in the other animals just mentioned.
3. Block produced by the method of cutting the A-V connection can be observed in all its transitional stages to complete recovery.
4. The cause of this block is believed to lie in the injury to the remaining fibers of the connection. This increases their refractoriness to receive and conduct stimuli. The strength of stimulus is decreased and its rate of conduction increased, until the impulse is completely blocked. The V fails to contract not because the impulse is too weak to excite it, but because the impulse does not reach it.

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SOME FEATURES OF OSTEOGENESIS IN THE LIGHT OF VITAL STAINING

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Since the time of Kölliker it has been customary to ascribe the erosion of calcified cartilage and provisional bone during endochondral ossification to the activity of a certain multinucleate cell known as the osteoclast. Although the exact manner in which this cell carried on its function, whether chemical, physical or mechanical, was obscure, it seemed possible—even probable—that the cell might be regarded as a phagocyte.

Now Goldman (1) has called attention to the reaction of the pathological giant-cell of experimental tuberculosis to certain dyes belonging to the benzidine group of colors; and has pointed out the intense staining of these cells following the introduction of dye solutions into the circulation of the infected animal. The work of Evans and Schulemann (2) showed that the staining phenomena which follow the exhibition of these benzidine dyes are closely related to—indeed are probably due to—the exercise, by certain specialized cells, of a phagocytic potentiality. These phagocytes, although anatomically heterogeneous and scattered about the body, are united by a common physiological activity in a great group. To designate them Evans borrows the term "macrophages" (Metchnikoff), and it is now recognized that their behavior toward trypan-blue and other dyes of the same class is merely the expression of a "scavenger" function which they hold in common, and which causes them to react to the ultramicrons of the dye-solution in the same way that they do to other débris which they ingest. Aside from all disputed questions concerning the histogenesis of the pathological giant-cells, the avidity with which they eat the vital dye-granules places them in the category of macrophages, and demonstrates the phagocytosis of which they are capable. If any further evidence were needed to establish their right to a place in this tissue-group the presence in their cytoplasm of bacilli or foreign bodies, which cause the lesion of which they are a part, is sufficient to ensure their classification as able and greedy phagocytes.

The anatomical similarity between these pathological elements and the normal osteoclasts of the bone-marrow, together with the supposition of a destructive action (possibly phagocytic) on the part of the latter cells, led us to expect, in the osteoclasts of animals whose bone and cartilage was undergoing rapid erosion, an ingestive reaction, identical with that of the macrophages, toward the high molecular dyestuffs and colloid solutions. And this reaction was looked for in spite of the fact that the cytoplasm of the osteoclasts shows none of the evidences of phagocytic activity in the form of ingested pigment or red blood cells, such as are found in the macrophagic reticulo-endothelium of the bone-marrow and the other fixed phagocytes of the body. It is perfectly possible to think of these cells as able to drink in the ultramicrons of colloidal or semi-colloidal solutions (in other words the finer material subdivisions), while, because of the coarser physical state of the substance, or for other reasons, they are not able to ingest the masses of large size which, enclosed in the cytoplasm of a cell, are the usually accepted evidence of its phagocytic powers. The idea therefore presented itself of staining young and growing animals with azo dyestuffs and metallic colloids and studying the young osseous tissue; a report of such a histological study is presented in this paper.

The method used was to inject into the peritoneal cavity of young and healthy animals (rabbits, kittens and chicks) a small quantity of a sterile aqueous solution of dye or colloid, using the technique and precautions described by us in a former paper (3). The animal was allowed to live for from one to four days and was then killed and the tissues fixed in 10 per cent neutral formalin. The fixative was injected into the blood-vessels, after which the tissues were allowed to stand in it for from twenty-four to forty-eight hours. They were then thoroughly washed, dehydrated, decalcified and embedded in celloidin or paraffin.

In our former communication (3) we have described the appearance in gross of the bones of such an animal, and have suggested their value in the study of ossification centers and the development of bone, and we shall speak of gross material only briefly here.

We shall also confine our description to the findings in animals injected with trypan-blue, an azo-dye, since the color of material stained with this dye is almost unchanged by passage through the strong acids used to decalcify the bony tissue in preparation for sectioning, and because there is no essential variation in the distribution of different benzidine dyes introduced into the body of an experimental animal.

The stain of the growing bone is in a large measure selective, resembling somewhat the osseous staining consequent upon the ingestion of madder; and while the bony skeleton is stained a very dark blue, the liver and spleen of these young animals are but lightly colored. This latter feature is especially marked in the chick. In fully developed adults exactly the reverse is true: the osseous system shows very little visible color, while the liver and spleen are the most heavily loaded places of dye-storage in the body. It would seem that the actively growing skeleton had taken to itself a large share of dye which, in the adult, would be cared for by these other tissues.

In a way this specificity of staining is what we might logically expect to find in these young animals. In them the growth curve of the skeleton is at its maximum height and the metabolism of the component elements of the osseous system must be whipped to the utmost. A study of the phenomena of vital staining demonstrates that circulating dye is always concentrated in loci of heightened metabolism, especially if, as is the case with the growing bony skeleton, the destructive or catabolic side of the life processes is prominent in the affected tissue. Thus we see the selective staining of the uterus during pregnancy, the heavy dye-deposits in the ovary which mark the situation of ova in the process of attrition, and, under pathological conditions, the special foci of dye-storage in inflammatory areas and about the borders of experimental neoplasms.

The osseous tissue is stained very darkly, and it is noticeable that this color is much more marked in the growing than in the fully developed bone. Indeed, upon close examination it is seen that especially dense staining marks the actively growing areas of the bone, as for example the ventral ends of the ribs, or the advancing borders of the membrane-bones of the skull.

These most deeply stained areas may or may not represent the so-called primary ossification centers. In fact the primary centers are stained only if they are areas of osteoblastic activity at the time of the exhibition of the dye. This is most evident in the thin bones of the developing skull where the primary centers are almost without color and the rapidly advancing edge of the membrane-bone is heavily stained.

It seemed, then, that the avidity with which the dye was taken by the growing bone had some connection with osteogenesis; and it was with the greatest interest that the sections were examined, for it was expected that the osteoclasts would be found loaded with blue granules. Instead of this it was surprising to find that no blue at all was to be seen in their bodies.

In other words these cells are non-trypanophilic and non-phagocytic. They can therefore not be classed with the pyrrhol cells on a physiological basis.

It may be objected that the fact that the osteoclasts do not store trypan-blue does not prove them non-phagocytic, since the ordinary neutrophil leucocytes—well known phagocytes—are not trypanophilic. These latter cells however show the commonly recognized signs of phagocytosis and are able to surround bodies whose size brings them easily into the field of microscopic vision. No such evidences of phagocytic activity are ever found in the cytoplasm of the osteoclasts, though they are present in pathological giant-cells, both of the foreign body and the granulomatous type.

If the osteoclasts have any part in the resorption of calcified cartilage and bone, that part must be played either through the pressure of their bodies against it or the production of a secretion which can dissolve or at least disintegrate the calcareous tissue with which it comes in contact. If the erosion of cartilage and bone is accompanied by phagocytosis of disintegrated tissue-fragments, that operation must be carried on by other elements in the marrow-cavity.

We are probably therefore justified in including the osteoclasts in the same category with the other giant cells of the bone-marrow, all of which are trypanophobic.

The only cells which manifested the characteristic trypanophil reaction were the ordinary reticulo-endothelial cells of the marrow reticulum. Though these were everywhere present throughout the marrow cavities of the young bone, they seemed to be most numerous in the areas where erosion of cartilage and bone was going on most actively. At the margin between cartilage and bone at the epiphysis there was a narrow zone where these macrophages were very numerous, and a distinct line of them could be made out along the edge of the cartilage to which they were apparently closely applied. In a region of the new endochondral bone, just behind the advancing osseous border, viz., in the region where the new spicules of endochondral bone were being most actively resorbed, they were more thickly grouped than in the rest of the marrow-cavity.

By recourse to a method of clearing the bones the distribution of the macrophages was made even more evident.

Bones were thoroughly dehydrated in ascending alcohols and, after two changes of absolute, were immersed in benzol. After remaining in this twenty-four to forty-eight hours they were drained quickly and put into oil of wintergreen. A striking picture is then presented by such a preparation. The color of the bone becomes much darker, almost black, and the separate centers of osteogenesis are sharply marked off from one another. In a long bone, for instance, the diaphysis appears as a blue-black shaft, the thin outer shell of periosteal bone being semi-translucent. At the ends the cartilaginous caps are seen to be almost completely unstained, and the epiphyses and apophyses, where these are present, appear as distinct nodules within their capsules of cartilage. So distinct is this demonstration of the foci of osteogenesis that they may be readily studied in preparations made by this method.

If the bones be decalcified before being cleared the picture is even more striking. The density of the blue is less marked, for the bone has lost its opacity because of the extraction of the calcium-salts, and, too, a small quantity of dye appears to be lost with the inorganic material. When such a preparation is viewed through the dissecting microscope or the binocular the distribution of the dyestuff is easily studied. In bones where the margin between cartilage and advancing bone forms a plane surface, as at the ventral extremities of the ribs, a distinct blue disk appears at this situation, and, on examining the specimen with a higher power, it is seen that the blue is within pyrrhol-cells or macrophages. They are met with throughout the area of endochondral ossification, but are specially abundant just behind the advancing tip, i.e., where new bone is being most actively broken down.

Some of the deep blue stain of the growing skeleton is due however, not to granules of color in specific cells, but to a diffuse deposit of dye in the bone itself. The greatest amount of bone so stained occurs in regions where bone-formation is going on most rapidly, at the growing ends of the bone, just beneath the periosteum on the shaft, and along the borders of spicules of endochondral bone in a narrow band just beneath the covering layer of osteoblasts. The largest and most deeply stained layer in the body occurs at the ventral costo-chondral junction—a focus of very rapid osteogenesis. The corpuscles embedded in bone so stained usually have their nuclei deeply and diffusely colored and occasionally granules of dye may be seen in their cytoplasm.

The meaning of this finding is not at all clear. Diffuse tissue-staining by solutions of benzidine dyes is usually accepted as evidence of tissue-injury, and nuclear coloration as certain evidence of cell-death. (McCurdy and Evans (4), Evans and Crowe (5), Evans Bowman and Winternitz (6)).

This staining, however, does not seem to be due to diffusion of the dye, and being in bone which has been freshly laid down, seems rather to be regarded as the result of the activity of the osteoblasts, some of which may be occasionally seen, faintly stained. It is probable that this stained bone was laid down during the time that the circulating body-fluids were charged with dye, and the stain may be comparable to the coloration of young bone in animals which are on a madder-diet. Schuleman (7) has shown that certain, and perhaps all, of the colored granulations formed in the pyrrhol-cells after vital staining with azo-dyes are the result of aggregation of the ultramicrons of the dye-solutions in the presence of electrolytes. It may be therefore that the coloration of the new formed bone is caused by the electrolytic precipitation of the injected dye, by and with calcium-salts, which have been locally concentrated to be laid down in the process of ossification. The question is being investigated further however, and the results will be published in a later communication.

The fact that the reticulo-endothelial cells are present in large numbers where absorption of bone and cartilage is going on, and that they are most numerous where this process is most active, is an indication that they are concerned in this work. We know that they are specialized to take into their cytoplasm small foreign bodies, as red blood-cells and bacteria (Kyes (8)) and pigment (Brass (9)), etc., and it seems reasonable to assume that they deal with the fragments of cartilage and bone, which are undergoing resorption, in the same manner. Whether or not these cells themselves actually break up the material is not clear, but if they do not at least it seems probable that they pick up the chips, so to speak, which other cells have produced.

What the pyrrhol-cells do with the matter which they have ingested forms a subject for fascinating speculation. They may digest it—break down complex into simple material—to be afterwards passed into the blood-stream and again utilized, or they may act as hod-carriers as well, transporting the bricks of calcium-compounds to regions where they can be built into new spicules of bone. Or they may simply prepare worthless materials so that they may be easily excreted.

If the pyrrhol-cells take up the material which has been formed from resorption of cartilage and bone, and there seems to be little doubt that they do, this suggests that such material occurs in a very finely dispersed form, since the macrophages are so specialized as to deal most easily with physical systems of this character.

CONCLUSIONS

1. The pyrrhol-cells occur in regions where bone is developing, and are most numerous in areas where the resorption of bone and cartilage is most active. It may therefore be considered that they take some part in this process, and it is believed that they ingest the débris and assist in clearing it away.
2. The osteoclast is not trypanophilic and hence must be excluded from the class of pyrrhol-cells and their derivatives such as the foreign-body giant cell.
3. Any office of the osteoclasts in the resorption of calcified cartilage or bone must be performed by the agency of chemical solution or mechanical erosion through pressure exerted by them upon the material which is being broken down.

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THE STAINING OF AMPHIBIAN LARVAE WITH BENZIDINE DYES WITH ESPECIAL REFERENCE TO THE BEHAVIOR OF THE LYMPHATIC ENDOTHELIUM

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A new point of view has developed during the last decade concerning the inter-relationship of certain cells of the blood and connective tissue; we have had no satisfactory classification for such cells heretofore. A number of morphologically heterogeneous endothelial and connective tissue elements have been grouped together into one class by the possession of a common physiological function—that is, the ability to ingest, aggregate, and store in their cytoplasm the submicroscopic particles afloat in colloidal sols and solutions of the acid azo (benzidine) dye-stuffs. To cells having this power Goldmann (1) has given the name pyrrhol-cell. Evans and Schulemann (2) have shown that the storage of the high molecular dyes by these cells is merely a manifestation of an ability to ingest and store foreign material, existing in the form of fine physical subdivision.

The pyrrhol-cells of the adult organism are of three general types. The first type, those which are truly endothelial in nature, line the capillaries of the liver, the venules and sinuses of the spleen, the haemal-lymph nodes, and the true lymphatic glands. The second type, which are more or less fixed cells, includes those widely distributed elements designated variously as adventitia, resting wandering cells, or clasmatocytes; and those colonies of cells closely akin to them, which are found in the omentum in which situation they are referred to as taches laiteuses. To this group belong also those interesting elements designated as reticulum-cells, which occur in the pulp of bone-marrow, spleen and lymph-glands. Thirdly, we find the free macrophages, which consist of the host of round, mono-nuclear cells inhabiting the serous cavities, the mono-nuclear cells occurring so abundantly at times within the lymph-sinuses of the lymphatic nodes and occasionally in the spleen and liver; and which under experimental conditions are found in large numbers in the latter places and even in the peripheral circulation.

The histogenesis of these various elements has become, with our increasing knowledge of their importance in the adult economy, a fascinating and tempting problem for anatomists. With the discovery of the special affinity of these cells for the diazo-dyes, it seemed as though we were on the threshold of solving the mysteries of the origin of blood and endothelium, and their relation to mesenchyme and the adult connective tissue types.

Technical difficulties soon arose however, and our dream still remained unrealized. The placenta was shown to be impermeable to vital dyes, and the direct injection of dye into the embryo to be impracticable. Introduction of the dyes into avian embryos was fraught with even greater difficulty, and repeated attempts have ended in failure. There remained no alternative but to try to introduce the dye by some means into the embryos of one of the cold-blooded vertebrates.

The object in attempting to stain amphibian larvae would be: to determine the fate of benzidine dyes when introduced into the body of the embryo, to discover which elements possess the power to phagocytise high-molecular particulate matter and to ascertain the distribution of these elements; and furthermore to show what relation these embryonic phagocytes might bear towards the pyrrhol-cells of the adult. Staining these embryos might assist us to a knowledge of the genetic interrelationship of the sessile pyrrhol-cells, and the wandering phagocytes of the mesenchyme and the serous cavities, or to shed light upon the embryology of types of cells (Kupffer cells, taches laiteuses) concerning which we know practically nothing.

Amphibian larvae, immediately after hatching, seemed suitable subjects for such an investigation although feeding experiments on the adult animal have led us to expect very little from absorption of the dyes through the alimentary tract, and the delicate organisms seemed unfit for such drastic measures as injection of the vital stain into the peritoneal cavity. So the most reasonable method appeared to be, to introduce the dye-stuffs into the water in which the larvae swam, and trust to its being absorbed through the respiratory tract or through the epidermis, which in amphibia is known to be very permeable.

For this purpose, eggs, of the common species of frogs and salamanders in the vicinity of Baltimore, were collected in April, 1916, and brought to the laboratory where they were cared for in balanced aquaria.

The species used were Rana temporaria, Hyla pickeringii and Amblystoma. The main experiments were carried out with Rana temporaria, repeated trial having demonstrated a greater immunity to the toxic action of trypan-blue, the benzidine dye used in the experiments, for this than the other two species. The author wishes to emphasize here, that trypan-blue is not the physiologically inert, non-toxic substance some writers have claimed, but that even comparatively weak solutions are definitely toxic, as will be clearly brought out by the following experiments. I have also found the dye to be definitely toxic to fish and adult amphibia when given intraperitoneally in 1 per cent solution with perfect technique.

Immediately after hatching, the larvae were transferred from the aquaria to finger-bowls containing graded strengths of freshly prepared trypan-blue solutions, made up with ordinary tap-water. In the initial experiment the strongest solution was 1 : 50. The others in decreasing strengths were: 1 : 100; 1 : 200; 1 : 400; 1: 800; 1 : 1600; 1 : 3200. The larvae were observed twice daily, morning and evening, for a few moments, as a routine, on a glass-slide in a small amount of water. The animals in the 1 : 50, 1 : 100 and 1 : 200 solutions were all dead and partly macerated the morning following the initiation of the experiment. Nearly all the larvae in the 1 : 400 solution were dead and the remainder died within the next twenty-four hours. After repeating the experiment with these strengths, the use of a solution more concentrated than a 1 : 600 was abandoned. In the 1 : 800, 1 : 1600 and 1 : 3200 solutions the animals remained alive indefinitely from two to four weeks and seemed to be little affected by the toxic action of the dye.

On the fourth day of immersion of the animals in the 1 : 800 and 1 : 1600 solutions, faintly discernible traces of trypan-blue were to be seen, in certain cells in the tail. On the fifth and the sixth days it became apparent that the trypanophilic cells, which were gradually becoming stained, lay either immediately adjacent to the course of the lymphatics, or were the cells of the lymphatic endothelium themselves. Observation of the living tadpoles on the seventh and eighth days, in a chamber such as described by Clark (3), left no doubt, but that the trypan-blue granules were within the cytoplasm of the endothelial cells of the lymphatic capillaries. Under the low power of the microscope the lymphatic system of the tail, including the caudal trunks, was brilliantly outlined in blue.

Under the high power of the microscope, in a Clark chamber, the nuclear areas of the lymphatic endothelial cells were visible at irregular intervals along the capillary wall. These areas appeared relatively free from trypan-blue, the dye corresponding in its distribution in the cell with the granular zone of the cytoplasm; and extending in both directions, it gradually faded away into the delicate strand of protoplasm outlining the wall. The vital dye was of uniform intensity, and the granules equally numerous, no matter whether the cell was situated near the sprouting tip of a growing vèssel, or lining an established channel.

After careful observation it became evident that the lymphatic endothelium had been stained quite specifically. The endothelium of the blood-vessels, many of which were distinguishable because they contained rapidly circulating blood-corpuscles, showed not the slightest particle of vital stain. Careful examination, of both mesenchyme and wandering-cells, for the slightest trace of the blue dye, was in vain; the specificity of lymphatic endothelium for the dye was complete.

It is evident, that, in the specific affinity of lymphatic endothelium in amphibian larvae, we have a means at our disposal for settling conclusively the long controversy, concerning the growth of lymphatics, in which studies on the lymphatics of amphibian larvae have played such a prominent röle in the past.

It may not be out of place here to discuss briefly the conflicting ideas about the mechanism of the growth of lymphatic vessels, which have been advanced and are now championed, by the modern students of angiology. Three views have assumed the most important places among the numbers of theories which have been evolved to explain the development of new lymphatic vessels.

1. That lymphatics may result from a transformation of blood-vessels, and that extension of the lymphatic system results from the coupling on or addition of these changed blood-channels.

2. That the endothelial cells of the lymphatics may arise from cells of the extra-vascular mesenchyme.

3. That after the initial outgrowth of lymphatics from the veins, in the form of lymph-hearts has occurred, neither mesenchyme cell nor blood-vessel endothelium contributes any further to their growth; but that they are an independent tissue, growing by division of primary lymphatic endothelium, and invading the embryonic body, as a vine climbs a trellis.

These three views, as they apply to the tadpole, can be subjected to a crucial test in the light of the discovery that trypan-blue is an elective stain for amphibian embryonic lymphatic endothelium. Experiments necessary to this end are being prosecuted in this laboratory. Even at the present time, while these experiments with vital dyes are only in their initial stages, it is possible by their help to draw certain fundamental conclusions, which may aid in directing the study of lymphatic growth.

For example, the results of the application of this method show that the Mayer-Lewis anlagen are not modified blood-capillaries but true lymphatics, since they store vital azo-dyes in their endothelium. This is in accord with the knowledge gained from the injection and reconstruction methods, which make it seem probable that the Mayer-Lewis anlagen are parts of a continuous lymphatic vessel.

The view maintained by a second group of writers, namely, that lymphatics grow by the conversion of extraintimal and perineural spaces into lymphatic channels, by the transformation of mesenchyme into endothelium, clearly becomes untenable, since mesenchyme and lymphatic endothelial cells are shown by their reaction toward the dye to be biologically different; and conversion of one into the other becomes extremely improbable.

The observations made so far upon vitally stained larvae, substantiate and add additional proof to the interesting observations by E. R. Clark (4) on the growth of lymphatics in the tadpole's tail. The author is able to confirm Clark's view that these lymphatics grow only by sprouting, and by sending out fine protoplasmic processes, which gradually become definite lumen-containing sprouts. Furthermore the author also agrees with Clark in maintaining that blood-vessels, and mesenchyme and wandering-cells, never contribute to the formation of lymphatic endothelium in this locality; but that each of these tissues has an independent, characteristic existence.

The staining of the larvae became so intense by the eighth day that even to the unaided eye a blue color was evident in the tail. A diffuse blue stain of the peritoneal cavity became apparent about the third day; but it seemed plausible to attribute this to the presence of dye in the intestinal contents, especially as a plug of faecal material stained deep blue could always be seen in the anal gut.

The animals were fixed at successive intervals from the third until the tenth day in 10 per cent neutral formalin. The material was dehydrated, imbedded in paraffin, cut, and stained with Mayer's carmalum.

Study of the sections seems to show conclusively that the epidermis does not serve as a portal of entry for the dye, since there are no visible traces of trypan-blue within or between the cells of this tissue. It seems more probable that the stain gains admission to the body through the alimentary canal, because in certain regions of the gut, the entire intestinal mucosa and submucosa are colored a deep diffuse blue and occasionally definite granules of dye are distinguishable in these layers. The embryonic intestine behaves radically differently towards the dye than does that of the adult, which under no circumstance, has been known to absorb the dye or to have visible particles of stain in the cells of the mucosa. Large amounts of the blue are aggregated in the cytoplasm of the vacuolated, mucoid epithelial cells, which line certain regions of the gill-pockets (notably those adjacent to the respiratory mucosa) and it is interesting to note the rich supply of lymphatics in this region, all of whose endothelial cells are loaded with trypan-blue. The respiratory epithelium appears to be practically free from trypan-blue granules, although in some instances areas may be found in which these cells have become actively phagocytic towards the stain.

As yet it is impossible to state positively whether the staining of the intestine represents an attempt on the part of the organ to absorb the dye, or whether this coloration is evidence of an excretion process or whether, as seems most probable, the absorption and excretion of the dispersed dye are not proceeding simultaneously through the intestinal epithelium. However, the mucous nature of the epithelium of the gill-pouches makes it seem probable that in this region we may be dealing with the excretory phase of vital staining.

A distinct attempt on the part of the body to excrete the dye, is evidenced by the remarkable appearance of the kidneys. The epithelial cells of many of the tubules are uncolored or contain only traces of the dye, whereas in other tubules the cells seem loaded to their full capacity. It is possible to find normal cells in all stages of transition between the two conditions. In still others there are degenerative changes, where judging from the accumulated stain, the cells are apparently taxed by the tremendous influx of foreign material, beyond their physiological limit. The epithelium in this instance is swollen to three or four times its normal height, the lumen of the tubule nearly obliterated, and the cytoplasm filled with clumps of dye and numerous vacuoles of varying sizes. It is not improbable to suppose, that the toxic action of stronger solutions of diazo-dyes upon poikilothermal vertebrates, can be accounted for by an identical, but more extensive degeneration of renal parenchyma, following an overtaxing of the kidney.

It is clear from microscopic study of the sections that the dye has been stored by three types of tissue as follows: by the lymphatic endothelium in its entirety, the Kupffer cells lining the sinuses of the liver, and by groups of large, round, mono-nuclear pyrrhol-cells occurring in the mesentery and omentum, not unlikely homologues of the taches laiteuses of higher vertebrates.

There is no phagocytic activity shown toward the dye by any type of cell elsewhere in the organism. There are no trypanophylic clasmatocytes in the connective tissue, nor are there any free "macrophages" discernible, either in the serous cavities, or in the blood-stream. There is no vital stain in the spleen or blood-forming tissue, and the circulating blood-elements do not contain a trace of the dye.

The lymphatic system throughout the organism is conspicuous by the brilliant trypan-blue granules displayed in the cytoplasm of its endothelium. Here, as in the living specimens, there is no difficulty in distinguishing lymphatics from the blood-vessels because of the absence of dye in the endothelium of the latter.

The nuclei of the lymphatic endothelium are small, flattened, lense-shaped, or somewhat irregular, and stain very deeply with carmalum; they never contain dye-granules, while the cytoplasm is conspicuous by the quantity of vital stain which it exhibits. The dye however appears to be in larger clumps and masses than in the living cell, where it is distributed in much finer and more diffuse particles. Besides the trypan-blue the cells contain fine pigment-granules, which are also visible in vivo, and which would appear to be normal for this species. The vital stain is limited in the stained section, as in the living cell, to the granular zone surrounding the nucleus, and does not extend far into the finer protoplasmic filaments of the sprouting tip of the lymphatic vessel, or into the strands of protoplasm extending from nucleus to nucleus and forming the capillary-wall. One receives the impression here, as well as in the living, that the dye granules have a tendency to remain near the more physically inert nucleus, rather than to enter into the plastic and markedly amoeboid pseudopods of the cell.

The liver is characterized in all the sections studied by the large quantity of dye which it contains. Here it is the endothelium of the vascular sinuses, the Kupffer cells, which house the dye. In the amphibian larvae the number of these cells relative to liver-parenchyma would appear to be very great. The liver cells themselves contain appreciable amounts of dye, a phenomenon seen in other animals at times; and possibly attributable to the fact that these cells absorb some of the dye in the presence of an excess in the blood-stream, which cannot be cared for by the cells, whose chief function it is to phagocytise particulate matter.

The third place of storage of the dye-granules, in the larval amphibians, is in the colonies of pyrrhol-cells in the mesentery and omentum. Some of these cell-aggregations seem to be true homologues of the taches laiteuses of higher vertebrates, others are merely accumulations of primitive wandering-cells peculiar to amphibian larvae in this locality. These cells are round and relatively large, having a single, round, eccentrically placed nucleus. Under no circumstance does the nucleus store the dye, but the cytoplasm, besides a quantity of brownish-yellow pigment-granules, displays an enormous amount of dye in clumps of differing dimensions and intensity.

The above results apply to all of the species studied, although the results with Hyla pickeringii were not nearly as satisfactory as with Rana temporaria, owing to the fact, explained above, that the toxicity of the dye towards the latter was very much greater, the animals succumbing before a brilliant vital stain was obtained. Amblystoma, although surviving longer than the other species in 1 : 1000 and 1 : 1200 solutions of the dye, had several-drawbacks, which militated against their use. For example, the study of the lymphatics in vivo was impracticable, although their endothelium has the same affinity for trypan-blue, because of the dense, opaque chromatophores and melanophores which characterize this species of amphibian, and also on account of the slow rate of absorption of the dye, the endothelium frequently showing no trace of dye before the twelfth or fourteenth day.

The question now arises as to the significance of this rather striking distribution of trypan-blue in amphibian larvae. Leaving out the intestinal tract, which is concerned only in the transmission of the dye to its final destination, and also the kidney, where there is merely an attempt on the part of the body to rid itself of an excess of foreign material, the only cells found storing the dye are the lymphatic endothelium (throughout its entire extent), the specialized endothelium of the liver capillaries, and colonies of pyrrhol-cells in the mesentery and omentum. May we regard the lymphatic endothelium of the amphibian larva as an intermediate stage in the phylogeny of the pyrrhol-cell? Could we but vitally stain the amphibian embryo at a still earlier period, it would not be startling to find the blood-vascular endothelium similarly stained. This is borne out by the fact that, after experimental injury to the blood-vessel wall, its endothelial lining-cells apparently resume a more primitive and embryonic function, as evidenced by the absorption of the vital-dye by the damaged endothelium. It would seem plausible, then, to infer that this phagocytic potential may be common to all endothelium in very early embryonic life, and that it may be lost progressively by the large majority of endothelial cells during their later development, and finally retained only by scattered groups of endothelial cells in the liver, bone-marrow, spleen and lymph-glands. What relation the fixed phagocytes in adult connective tissue bear to this primitive phagocytic endothelium is not at this time clear; but it is to be hoped, that the additional work now under way, on amphibian and other embryos, may eventually settle this much discussed question.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

II. The Influence of the Vagus Nerves on the Fractionate Contraction of the Right Auricle

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I. INTRODUCTION

It is generally conceded that stimulation of the vagus nerves causes a marked reduction in the amplitude of auricular contraction in mammals. Experiments similar to those reported by MacWilliam in 1888 (1) have been frequently reduplicated by experimental investigators. When the movement of a point on the auricular surface (usually the appendage) is transmitted to a recording lever or a tambour transmission system, the amplitude of the recorded curves becomes gradually smaller during weak vagal excitation until, in some instances, complete stoppage occurs. Upon cessation of stimulation the reverse process follows; the beats gradually increase in amplitude until a range equal to or exceeding the normal occurs.

In the foregoing paper (2) it was pointed out that not only does the "suspension curve" not represent a true record of auricular shortening, but the myogram recorded by an efficient myocardiograph from two approximating points is not necessarily an index of the contraction process as it affects the individual units of auricular muscle. Evidence was presented which favored the conception that the contraction process spreads over the auricle in the wake of the excitation wave in such a manner that the portions nearer the sinus node begin to relax before the more distal portions have ceased to contract. The interval that any unit of cardiac tissue remains in the contracted state was designated as the *fractionate contraction*. When the approximation of two distant points is recorded, the shortening, designated as the *mechanical contraction*, represents the algebraic sum of fractionate contractions and relaxations between these points.

It is evident that the influence of the vagus nerves on the contractile function of the auricular tissue can be satisfactorily tested only by utilizing the *fractionate contraction*. While it is impossible to obtain a record of the contracted state of a small imaginary unit of auricular muscle, it seemed probable that such a record of the fractionate contraction could be approximately realized by attaching the arms of the miniature myocardiograph to points only 3 or 4 mm. distant on the auricle. Such proved to be the case.

In figure 1 are shown two exactly superimposed records of the mechanical contraction (lower curve) recorded from points 18 mm. apart on the anterior surface of the right auricular and the fractionate contraction (upper curve) recorded for two points 2.5 to 3 mm. apart near the distal arm of the other myocardiograph attachment. The two curves show distinct differences in contour. The myogram of the fractionate contraction differs from that of the mechanical contraction in the following respects:

1. The onset is delayed about 0.02 second (e.g., point A).
2. The gradient of the descending limb is straight.
3. The apex is acute.
4. The ascending limb (relaxation) coincides with that of the mechanical contraction.

II. EXPERIMENTAL RESULTS

The right and left vagus nerves were stimulated in a total of 26 experiments. The strength of current was so adjusted that a moderate retardation of the auricle was induced.

Vagus excitation increases the amplitude of the first vagal beat but progressively decreases the amplitude of succeeding beats. This statement applies alike to the mechanical and fractionate contractions, as illustrated in figure 1. The increase in amplitude of the first vagal beat, which appears not to have been noted by less sensitive methods, was present without exception when a moderate rate of retardation occurred. This increase is striking when a long diastolic interval precedes the first vagal beat but requires measurement to discover when the cycles lengthen very gradually. It is entirely absent, as shown in figure 2, when the rhythm during vagus excitation remains unaltered. This shows that a considerable latent period elapses before the depressing effect of vagus excitation becomes predominant over some other influence tending to augment the contraction. What this influence is—whether due to the beneficial influence of the longer

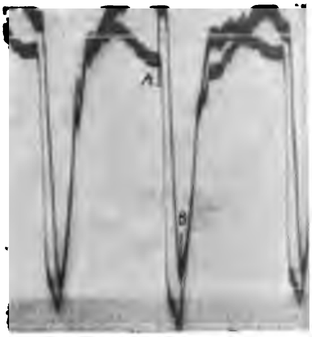

Fig. 1. The fractionate contr

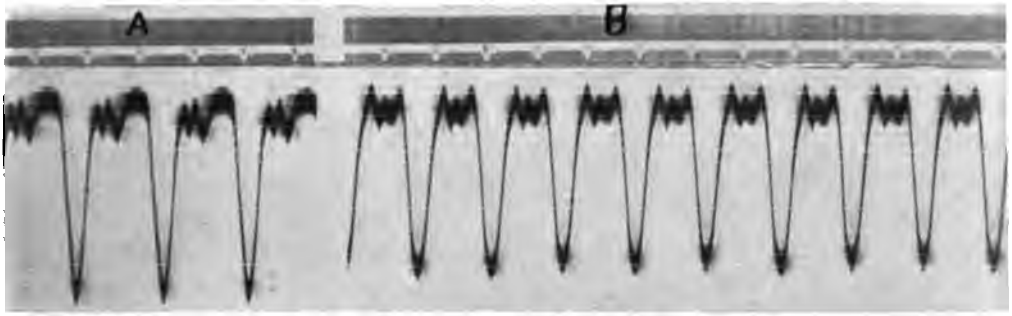

Fig. 2. *A*, Record of normal fractionate contraction of right auricle.

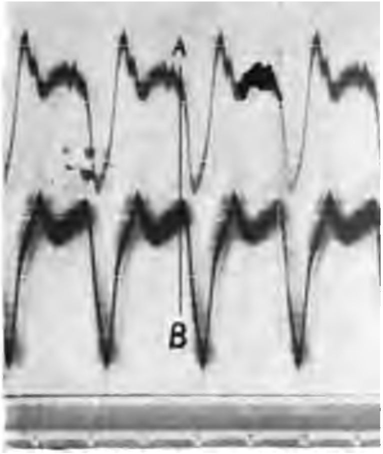

Fig. 3. Two records of fractionate c

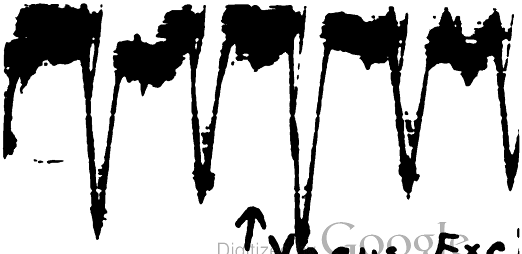

Fig. 4. Record of the fractionate contraction of

rest period which precedes, or to the simultaneous stimulation of antagonistic nerve fibers in the vagus—can not be absolutely determined from these experiments. The fact that it is dependent upon the lengthening of a preceding diastole strongly favors the former view.

Figure 1 shows that after the first vagal beat the amplitudes of the mechanical, as well as of the fractionate contractions, progressively decrease. Hence, it may be assumed that the decrease in the amplitude of the mechanical contraction is due to a corresponding decrease in the contraction of each fractionate part of the auricular muscle.

The depressant effect on the contraction of the right auricle is exerted by both vagi, is independent of any dromotropic action, but is always associated with variations in rate. In figure 3 of the previous article, the effect which stimulation of the left vagus produces on the mechanical contraction is shown. Auriculo-ventricular block was present, showing that this nerve exerted a marked dromotropic action on the *a-v* bundle at the time that the depression of auricular contraction occurred. A comparison with figure 1 of this article, where no dromotropic action is evident, shows that the depressant action is exerted equally by the two vagi and can not be secondary to changes in conductivity. Following the lead of Pawlow (3) and others, an attempt was made by stimulating various intrathoracic branches of the right vagus nerve to obtain an effect on amplitude independent of variations in rhythm, but no such dissociation could be elicited.

The depressant effect of vagus excitation persists when the rhythm of the auricle is maintained by regularly applied electrical stimuli. The first portion of figure 2 shows a series of normal fractionate contractions of the proximal end of the right auricle. The next section shows the fractionate contraction resulting when the normal pacemaker was supplanted by an artificial series of electrical break shocks given at a rate somewhat more rapid than the normal rhythm. Owing to the shorter diastole their amplitude is smaller. While this excitation was maintained the right vagus was stimulated. The customary reduction in amplitude of the artificially induced contractions occurred, showing that the variation in amplitude occurs independently of the chromotropic action of the vagus.

The depressant vagus effect is exerted equally and simultaneously on the fractionate contractions of the proximal and distal portions of the right auricle. Figure 3 shows simultaneous records of the fractionate contractions taken from the proximal (upper curve) and distal portion (lower curve) of the right auricle. The lines *AB*, representing synchronous points, show that contraction and relaxation of the proximal portions of the auricle precede that of the more distal portions. Vagus excitation in this instance is followed by two larger beats after which the characteristic depression in amplitude results in both curves. Recovery which, owing to the length of the record, can not be reproduced, followed in reverse fashion but synchronously in both curves.

Vagus excitation does not alter the duration of the fractionate contraction. On account of the sharp apex of the *fractionate* contraction curves there is no difficulty in establishing the precise onset and termination of auricular contractions. Careful measurement of curves, such as those shown in figures 1, 2 and 3, shows absolutely no variation in the interval of fractionate shortening during vagus stimulation. The gradient of the down stroke becomes, therefore, less steep. The mechanical contractions recorded from more widely separated points show small variations in the duration of contraction. Sometimes there is a lengthening and sometimes a shortening of the contraction periods, but as these variations do not exceed those normally occurring, they are apparently not significant. The reason for these variations can probably be found in the explanation advanced in the preceding paper—that the apex does not represent the end of all contraction processes, but a balance of contracting and relaxing fibers. As shown in figure 1, this balance shifts slightly during vagus excitation. In the normal beats previous to excitation the apex of the fractionate contraction falls beyond the apex of the mechanical contraction. In the first two vagal beats they coincide and in the third beat it falls on the ascending limb of the mechanical contraction. These results emphasize the importance of utilizing approximately fractionate contractions in studying the fundamental properties of the auricle muscle.

Vagus excitation reduces the irritability of auricular muscle but this can not account for the final reduction in amplitude. If the auricle is excited by a series of minimal break shocks applied at a rate exceeding the normal rhythm and the vagus nerve is then stimulated, these artificial stimuli may become subminimal, as shown by the fact that the auricle lapses into a rate equal to or slower than its normal. An indication of this is shown in figure 4. After vagus stimulation the amplitude decreases in the typical manner. The artificial tempo is at first unaltered, as in figure 2; later, as shown by the beats x, x the artificial stimuli become subminimal and slower beats occasionally appear. This in turn may be followed by a resumption of the normal or a slower rhythm. While such experiments show that the vagus depresses the irritability of auricular tissue, evidence is lacking that this depression occurs as early as the reduction in amplitude. Diligent efforts to demonstrate such a synchronous and early reduction of irritability have failed. As far as experimental evidence goes, it appears that the reduction of irritability follows the reduction in amplitude and is not responsible for it.

The auricular depression following vagus stimulation is independent of changes of initial intra-auricular pressure and initial length. In figure 3 of the preceding paper of this series is shown a record in which, during vagus stimulation, a gradual dilatation of the auricle accompanies the increase of intra-auricular pressure. The initial length of each successive beat increases, as shown by the distance of the points *A* from the base line. A similar result is shown in figure 2. As the combined increase in initial length and initial tension tends to increase the amplitude of contraction, it is clear that the reduction of amplitude has occurred in spite of these factors.

This is not the common effect of vagus stimulation on the initial length, as indicated by these experiments. In 17 out of 26 cases the initial length decreased shortly after the onset of stimulation (fig. 1) and an analysis of those tracings, accompanied by an intra-auricular pressure record, shows that it occurs in spite of an increase in initial pressure within the auricle. These cases all show the same gradual reduction in the amplitude of the mechanical or fractionate contraction of the auricle.

III. THE NATURE OF THE VAGUS INFLUENCE OVER AURICULAR CONTRACTION

The vagal depression of auricular contraction has been assigned to various influences. It is the prevailing opinion that it is not secondary to changes in the intra-auricular or the coronary pressure. MacWilliam (1) found the depression entirely independent of the degree of stasis or the height of intra-auricular pressure. This was confirmed by these experiments; in fact, when an increased initial pressure and decreased initial length occur one would expect these influences to augment rather than reduce the amplitude of contraction. Langendorff (4) found that the reduced amplitude occurred in perfused hearts without variation in coronary pressure and the writer obtained similar records years ago.

The most plausible view attributes the depressing action of the vagus nerve to a direct influence on the auricular muscle. The nature of this influence has been the subject of extensive experimentation and discussion. Does the vagus nerve depress the function of contractility directly or is this function inhibited as a secondary result of a primary alteration in the functions of rhythmicity, conductivity or irritability? The evidence in regard to the proposition that such secondary causes are concerned may be briefly reviewed:

1. That the reduction in contraction is secondary to changes in rhythm. The reduction in amplitude of the auricular contraction is usually associated with a decrease in rate. According to François Franck (5) and Bayliss and Starling (6), it is possible by vagus excitation to elicit one phenomenon without the other. This could not be confirmed in this research. When, however, the auricle was excited by electrical stimuli well above the minimal in strength, excitation of the vagus nerve produced a reduction in the amplitude without, of course, modifying the rate. These results show the complete independence of changes of rate and amplitude.

2. That reduction in contraction is secondary to changes in conductivity. As postulated by Englemann (7), a depression of conductivity may reduce the amplitude of contraction in three conceivable ways:

(A) The velocity of the excitation wave may be decreased so that the interval within which the proximal and distal units of auricular tissue enter into contraction, increases. In opposition to such an assumption Englemann cites the following evidence:

(1) No delay can be observed between the contractions of different portions of the frog's auricle.

(2) The duration of the contraction period in the frog is shorter and not longer during vagus excitation (8), (9).

Both of these observations have been contradicted, however, in the case of mammals; the former by Hering (10) who professed to *observe* that during vagus excitation the contraction wave progressed more slowly in the rabbit's auricle; the latter by Klug (11) who found that the duration of auricular contraction was longer after vagus excitation. Neither of these observations is corroborated by later work. Of more value than the observations of Hering because they were carried on by methods permitting greater accuracy, are the results of Lewis, Meakins and White (12). They found that the rate at which the impulse travels over the auricle is, to judge from the spread of the electrical wave, not modified by vagus stimulation. Klug, owing to the method employed, was not able to record the myogram of the auricle correctly. The evidence submitted in this paper shows that the duration of the fractionate contraction is unaltered by vagus stimulation.

(B) The excitation wave may be blocked so that it fails to reach the most distal portions of the auricle. In consequence, a shorter length of auricular tissue would contract and the amplitude would be reduced. This view has been discounted

(a) By the failure to observe a quiescent region in the more distal portions of the auricle (Englemann);

(b) By the fact that a negative variation does spread to the more distal portions (Lewis, Meakins and White);

(c) By the fact that separate records of the proximal and distal portions of a partially clamped amphibian auricle may show a decrease in amplitude in the distal segment during vagus stimulation without a corresponding decrease in the proximal region;

(d) By the fact, shown in this research, that the records of the fractionate contractions taken from the distal and proximal portions of the mammalian auricle undergo a simultaneous and proportionate decrease during vagus stimulation.

(C) Without any impairment of longitudinal conduction, the cross conduction through cardiac fibers may be impaired during the vagus stimulation. In consequence, a smaller cross section area would contract. The fact that widespread extraneous stimuli applied to the auricle during vagus excitation gives rise, not to a larger, but to a smaller contraction, is cited by Englemann against this possibility.

The evidence of experimental investigation is evidently opposed to the assumption that the reduction in amplitude of auricular contractions is secondary to disturbances of conductivity.

3. That the reduction in amplitude is secondary to changes in irritability. The observations of MacWilliam, Englemann and others, that stronger stimuli are required to elicit an extra contraction during vagus stimulation, are usually cited to show that in the mammalian and amphibian auricle a distinct bathmotropic effect is induced by vagus stimulation. The difficulty in applying this method consists in exciting the auricle in exactly the same phase of relaxation. More distinct proof may be gained by exciting the auricle by a series of minimal artificial stimuli. Upon vagus excitation, as shown by Englemann in the case of the frog's heart, and extended to the mammalian heart in this research, these stimuli become subminimal, indicating a reduction of irritability of the cardiac tissue. It was pointed out, however, that no experimental evidence of a reduced irritability could be obtained as early as the reduction in contraction amplitude. While the possibility that the decreased irritability following vagal excitation is, in part, accountable for the reduction in amplitude must be borne in mind, experimental evidence does not favor this explanation of the early reduction in amplitude.

By exclusion, we are forced to the conclusion that the vagus exerts a depressing influence on the contractility of auricular muscle. There is also direct evidence that such a negative inotropic effect exists. Englemann showed that when the frog's auricle is stimulated artificially at a rate exceeding that set by the pacemaker and variations in rhythm and irritability are thus avoided, vagal stimulation still decreases the amplitude of auricular contraction. This has also been shown to be true for the fractionate contraction of the mammalian auricle by the experiments reported above.

IV. CONCLUSIONS

1. The depression of the mechanical contraction of the mammalian auricle which follows vagal excitation is due to an actual decrease in the contraction of the individual cardiac units.
2. The decrease is not secondary to changes in rhythm or to alterations in conductivity.
3. The decrease may be favored by the depression of irritability produced by vagus stimulation but is primarily due to the depressant effect of the vagus on the function of contractility.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

III. THE TIME RELATIONS OF AURICULAR SYSTOLE

I. THE EVENTS OF AURICULAR SYSTOLE AND THEIR RELATION TO VENTRICULAR SYSTOLE

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In an earlier paper (1) the term "auricular systole" was restricted to the interval elapsing between the inception of contraction and the maximal shortening of the entire auricular musculature. Evidence was presented which showed why it is impossible to calculate this interval, either from the waves of the intra-auricular pressure curve or from the myogram taken individually. Auricular systole extends from the first rise of intra-auricular pressure to the apex of the myogram curve. Its duration averages roughly 0.1 second.

The interval elapsing between the end of auricular systole and the beginning of ventricular systole (determined from the rise of the intraventricular pressure curve) may be designated as the *intersystolic interval.*[1]

As shown in figure 1, this interval may be non-existent, the rise of the intraventricular pressure coinciding absolutely with the termination of the myogram curve. Under abnormal conditions, as shown in the fourth premature ventricular systole, the rise of intra-ventricular pressure may even precede the termination of auricular systole. As a rule, however, a short interval is found.

As shown by the experimental results in column 1 of table 1, the intersystolic period varies from 0 to 0.068 second, averaging 0.024 second. This shows that the contraction and relaxation of the mammalian chambers are so timed that very little or no time is lost between the termination of auricular systole and the inception of ventricular contraction.

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[1] In thus defining the intersystolic interval the writer is aware that the term is employed frequently but with little reason to designate the interval elapsing between the onset of auricular and ventricular systoles; i.e., synonymously with A_s–V_s interval.

The intersystolic interval added to the period of total systole gives the A_s-V_s interval; i.e., the time elapsing between the onset of auricular systole, as evidenced by the first rise of intra-auricular pressure, and the onset of ventricular systole, as evidenced by the first rise of intraventricular pressure. This period (column 3) ranges from 0.091 to 0.18 second and averages 0.130 second. Since the period of systole varies independently of the intersystolic interval, it is apparent that *the A_s-V_s interval is not an accurate index of the inter-systolic interval.* In column 4 of table 1 it is shown that the ratio between these two periods varies from 1 : 2 to 1 : 10.2.

TABLE 1

EXPERIMENT	1	2	3	4	5
	Intersystolic interval	Total systole	A_s-V_s interval	Ratio Intersystolic A_s-V_s interval	Interdynamic interval
	second	*second*	*second*		
C75 VIII	0.068	0.070	0.138	1 : 2	
C77 VI	0.020	0.126	0.146	1 : 7.3	
C78 III	0.011	0.08	0.091	1 : 8.2	
C79 IV	0.045	0.126	0.171	1 : 3.8	
C80 XV	0.063	0.077	0.14	1 : 2.2	
C84 I	0.040	0.14	0.18	1 : 4.5	0.014
C89 I	0.012	0.10	0.112	1 : 9.3	
C93	0	0.124	0.124		0.05
C96	0.010	0.102	0.112	1 : 10.2	
C97	0.028	0.14	0.168	1 : 6	
C98	0	0.108	0.108		
C100 II	0.014	0.130	0.144	1 : 10.2	
C103 XI	0.050	0.078	0.128	1 : 2.56	
C116 I	0	0.11	0.11		0.044
C124	0.002	0.09	0.092		0.025
C70 VI					0.075
C87					0.070
C88					0.013
C90					0.013
C91					0.038
C121					0.075
Average	0.024	0.106	0.13		0.042

The intra-auricular pressure continues to rise only during the early half of systole and declines during the latter portion. This was first pointed out by Ewing (2) and was confirmed by photographic tracings of the writer (1). The interval between the maximum intra-auricular pressure and the onset of ventricular systole may be designated as the *interdynamic interval* because any influence that intra-auricular pressure might have upon the initial intraventricular pressure steadily diminishes as the duration of this period increases. This interval is obviously longer than the intersystolic interval and in dynamic considerations is much more important. The results of column 5 in table 1 show that this period varies from 0.013 to 0.075 second, averaging 0.042 second. It is proportional neither to the A_s-V_s interval nor to the intersystolic period, as is evidenced by a comparison both of average figures and of the actual figures in the same case.

II. THE RELATION OF AURICULAR SYSTOLE TO THE WAVES OF THE VENOUS PULSE AND THE ELECTROCARDIOGRAM

In the experiments analyzed in this and a preceding paper a considerable variation existed, not only in the duration of various phases of auricular systole but in their relation to ventricular contraction. The possibility must always be borne in mind that this may be due to variable disturbances incident to exposure and cooling of the heart, abnormal circulatory derangements consequent upon an abolition of the negative intrathoracic pressure, etc. It would, therefore, be very desirable to obtain data in regard to the auricular systole and its relation to ventricular systole from unoperated animals and man. Inasmuch as the time relation of the venous pulse and electrocardiogram are often considered of value in such determinations, it is important to establish their relations to the dynamic changes occurring within the heart chambers.

In obtaining multiple records of different cardiac events by photographic projection, it is important, as has been many times emphasized (3), (4), (5), that the optical mirrors must be aligned in the same vertical plane if the records are to be precisely over one another at any moment. As this is sometimes difficult, proper corrections for displacements must be made by briefly exposing the paper while it is not moving. The relative point at which each record starts after opening the shutter may be used as a correction provided the photokymograph is so constructed that the paper is running evenly at the moment when the shutter is first opened and provided also that the shutter exposes all parts of the slot equally and simultaneously.[2]

Relation to the waves of the venous pulse. The time relations of the presystolic wave of the external jugular pulse recorded from the lower cervical region by a covered receiving tambour connected with a Frank capsule, are shown in figure 2. The jugular pulse consists of a presystolic wave *P*, a systolic impact *S*, followed in this case by a large after oscillation due to a light apposition of the receiving tambour. The second sound occurs at S_2 after which, owing to the short diastole, only a small diastolic wave follows. In this case, the onset of auricular systole, as indicated by the rise of intra-auricular pressure, preceded the rise of the jugular pulse 0.06 second. Considerable variation of this figure exists, however, as shown in table 2. The actual delay is probably largely determined by the distance from the auricle. When the pulsations are recorded directly from the superior vena cava the delay becomes very small.

TABLE 2

EXPERIMENT	TOTAL SYSTOLE	DYNAMIC INTERVAL	PRESYSTOLIC WAVE (RISE)	DELAY IN PRESYSTOLIC WAVE
	second	*second*	*second*	*second*
C75............	0.070	0.041	0.045	0.092
C77............	0.126	0.038	0.043	0.056
C78............	0.080	0.042	0.043	0.038
C79.	0.126	0.062	0.055	0.071
C96............	0.102	0.050	0.049	0.048
C121...........	0.100	0.037	0.038	0.060
Average.......	0.101	0.045	0.0455	

The duration of the rise of the presystolic wave is apparently shorter than the total systole but corresponds closely to the dynamic interval. This is shown by a comparison of the rise with the total systole, *x-y*, and the dynamic interval, *a-b* (fig. 2), as well as by the figures given in table 2.

Inasmuch as the summit of the presystolic wave (although delayed) corresponds to the summit of the auricular wave of the intra-auricular pressure, and as the latter does not represent the end of auricular sys-

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[2] The onset of the tracings is not indicated in the reproductions of this article owing to the fact that they were slightly shortened during the process of engraving.

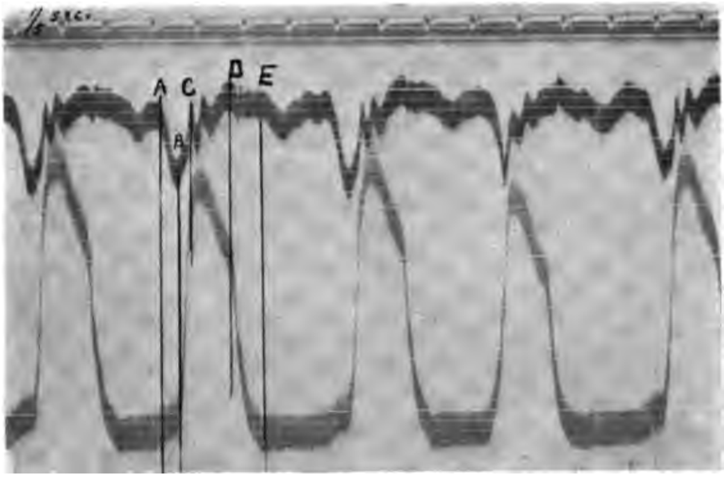

Fig. 1. Synchronous records of right auricular myogram and left intraventricular pressure. Points exactly superimposed. *B*, end of auricular systole; *C*, onset of ventricular ejection; *D*, end of auricular ejection; *E*, end of relaxation. Showing absence of an intersystolic interval and correspondence of changes between intraventricular pressure and accidental auricular movements. In the third beat a premature ventricular systole occurs before the end of auricular systole.

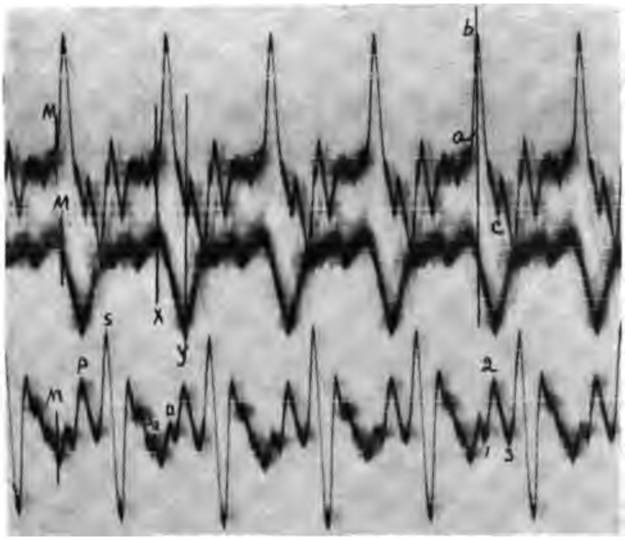

Fig. 2. Right intra-auricular pressure (upper curve); *a*, beginning of auricular systole; *a-b*, dynamic interval; *c*, systole variation. Right auricular myogram (middle curve); *x-y*, period of auricular systole. External jugular pulse (lower curve); *P*, presystolic wave; *S*, systolic wave; S_2, second sound; *D*, diastolic wave. *1, 2, 3*, duration of auricular wave. Lines *M* show displacement of points due to alignment of recording apparatus.

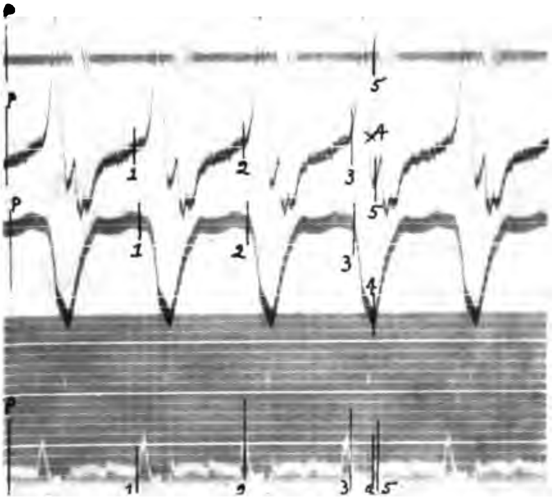

Fig. 3. Ventricular sounds (upper curve); right intra-auricular pressure (second curve); right auricular myogram (third curve); electrocardiogram, lead II (bottom curve). *P*, relative alignment of points. Numerals *1, 2, 3, 4, 5.* synchronous points on records.

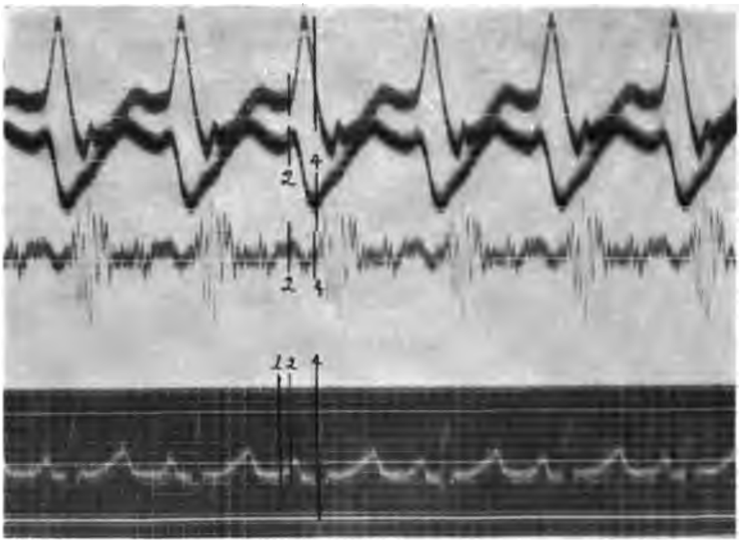

Fig. 4. Right intra-auricular pressure (upper curve). Right auricular myogram (second curve). Ventricular sounds (third curve). Electrocardiogram, lead II (bottom curve). Numerals same as in preceding figure. Time 0.05 second.

tole, *it is obviously impossible to estimate the intersystolic interval from the jugular waves.* Are we justified in using the interval extending from the summit of the presystolic wave to the arterial impact as an index of the *interdynamic interval?* To obtain equality it is necessary that the delay occasioned in venous transmission should be very nearly offset by the delay in arterial transmission plus the isometric interval. In figure 2 the interdynamic interval, *b-c*, equals 0.06 second; the equivalent period, 2-3, in the venous pulse also equals 0.06 second. Similar relations are shown in results arrayed in table 3. Evidently, the *a-c* interval averages about 0.015 second less than the true inter-dynamic interval. An approximation of the interdynamic interval may therefore be made only roughly in normal rhythms.

TABLE 3

EXPERIMENT	INTERDYNAMIC INTERVAL ESTIMATED FROM	
	Intra-auricular pressure	Jugular pulse
	second	*second*
C84	0.14	0.131
C88	0.13	0.096
C89	0.05	0.028
C91	0.038	0.048
C125	0.075	0.060
Average	0.087	0.0726

Since the rise of the presystolic wave very nearly equals the dynamic interval, the duration of the A_s-V_s interval may be approximated by the *a-c* interval. Unfortunately, this is of little scientific value because the A_s-V_s interval is not proportional to the intersystolic interval and its relation to the conduction time is questionable. The writer has elsewhere (6) discussed the assumption that must be taken for granted, especially in pathological cases when the A_s-V_s interval is used as a criterion of conduction time.

Relation to the waves of the electrocardiogram lead II. The relations of the intra-auricular pressure variations to the waves of the electrocardiogram taken by lead II have been recently reported by Garten and Weber (7). Their results will be referred to in comparison with those yielded by a consideration of nine experiments in which the auricular myogram was also recorded. Figure 3 shows such a record. The first electrical variation starts at 1. It precedes the rise of intra-auricular pressure, 2, by an interval of 0.022 second and the onset of mechanical shortening, 3, by 0.033 second. The former figure was duplicated or approximated in other experiments (cf. figs. 4 and 5) and agree with the results of Garten and Weber who report variations from 0.013 to 0.021 second.

The precise relation of the onset of the intra-auricular pressure rise to the *P* wave varies slightly. It may, as shown in figure 3, occur precisely at the summit of the *P* wave. This also accords with the tracings published by Garten and Weber. It was found more frequently, as shown in figures 4 and 5, to come somewhat earlier. While there is no precise relation between the mechanical systole and the *P* wave of the electrocardiogram, the summit of the *P* wave may, without great error, be taken as indicating the approximate onset of auricular systole.

The relation between the end of mechanical systole and the waves of the electrocardiogram is still more variable. In figure 3, auricular systole does not terminate until the completion of the *R* wave, 4, and this is followed after a short interdynamic interval, 5, by ventricular systole, as indicated both by the heart sounds and intra-auricular pressure curve. This, it may be emphasized, proved later than the average cases in which, as shown in figures 4 and 5, it terminated just before or after the peak of the *R* wave. According to these results, auricular systole does not terminate until ventricular excitation is well under way.

III. PHYSIOLOGICAL IMPORTANCE OF RESULTS

The data collected in this and the two preceding papers enables us to state with greater precision the details of the cardiac cycle.

About 0.02 second after an impulse is emitted from the sino-auricular node the right auricle begins to contract, as evidenced by the rise of intra-auricular pressure. The precise onset of this mechanical shortening can not be determined from the waves of the venous pulse but may be approximated by the peak of the *P* wave taken by lead II. This mechanical shortening is due to a series of fractionate contractions which spread across the auricle in the wake of the excitation wave. The auricle continues to shorten as long as the contracting units of cardiac tissue over-balance the relaxing units. As soon as an exact balance is established no further shortening is possible and the termination of the systole has come. This period lasts about 0.11

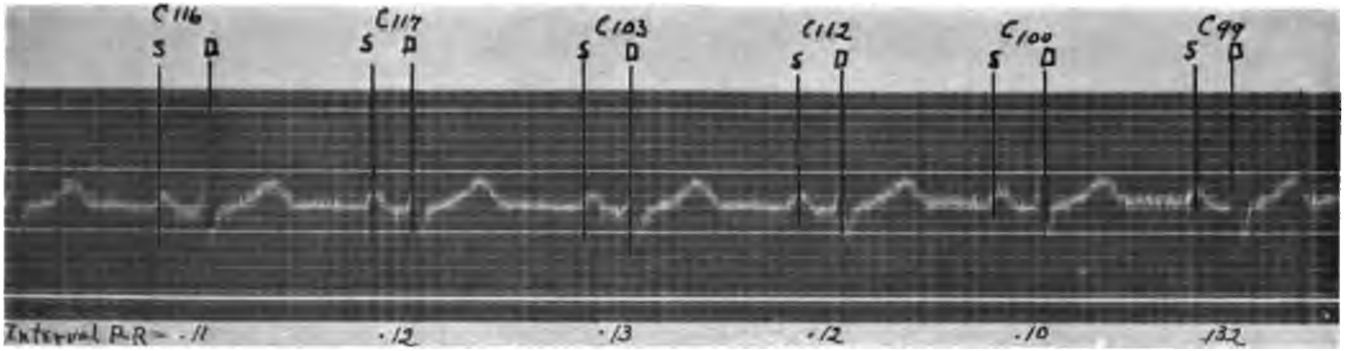

Fig. 5. A normal electrocardiogram, lead II, upon which have been marked for comparison the beginning (*S*) and end of auricular systole (*D*) as found in different experiments. At bottom, *P-R* intervals found in these cases are marked. Time, 0.05 second.

second. The contraction of the auricle exerts a dynamic action by elevating intra-auricular pressure. This occurs early in systole but continues for only 0.05 second; during the remainder of systole the intra-auricular pressure falls. This *dynamic interval*, as the period during which pressure rises is called, may be estimated from the rise of the presystolic wave of the venous pulse, *a*-wave, but the total duration of systole can be determined neither from the venous record nor the electrocardiogram.

The auricle continues to contract after excitation of the ventricle has occurred, as shown by the fact that it terminates during the *R* variation of the electrocardiogram. The ventricle normally never contracts until auricular systole is completed. Occasionally ventricular systole follows immediately but usually an intersystolic interval, averaging 0.024 second, intervenes. The duration of this interval can be accurately determined neither from the jugular tracing nor from the electrocardiogram. The *a-c* interval of the jugular pulse in normal and regular rhythms very nearly equals or at least varies with the A_s-S_s interval, but the latter is not a criterion of the intersystolic interval and does not vary with it.

It is evident from this work and that of previous investigators that the electrocardiogram is capable of showing (1) the time of emission of an impulse from the sinus node (P wave); (2) approximately the onset of auricular systole (summit of *P* wave) and (3) the conduction time between sinus and ventricle (*P-R* interval). The venous pulse gives neither the onset of auricular systole nor its duration but the dynamic interval of systole may be calculated from the rise of the presystolic wave.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

VII. VARIATIONS IN THE CHEMICAL COMPOSITION OF REPRODUCTIVE TISSUES IN RELATION TO VARIATIONS IN FUNCTIONAL ACTIVITY

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Tissues in which prolonged periods of great activity alternate with long periods of nearly complete rest are unusual in animals. Fully differentiated and functionally active tissues which undergo a permanent and sudden change from a very low state of activity to a much higher one are also uncommon in animals. Some of the reproductive tissues of birds are admirably adapted to securing tissue samples in which this contrast is most pronounced.[1] Such contrasting states of the tissue may here be obtained moreover without the least artificial stimulation or restriction of any of the habits, powers, or processes of the animal. The chemical differences observed in tissues so obtained should therefore be of considerable interest.

Mayer and Schaeffer (1) found that the tissues of some hibernating animals show seasonal fluctuations in their content of lipoid phosphorus. And, further, when the body temperature of warm-blooded animals is either raised or lowered to proper levels, the lipoid phosphorus shows marked variations, and these variations by no means always parallel the variation in total fatty acids. Finally, the work of Mayer and Schaeffer would seem, in the main, to indicate that *changes* in the physiological activity of the tissues (lungs, liver) studied are, in a majority of cases, associated with an *increase* of phosphatides in the tissue. The probable rôle of the phosphatides of the cell has recently been rather fully treated by Mathews (2, chapters 3 and 13).

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[1] A fact deserving consideration is that in animals the reproductive system, of all the systems of the organism, is the most easily, and most profoundly affected by environmental and intra-organic change. Darwin made the following observation: "Many facts clearly show how eminently susceptible the reproductive system is to very slight changes in the surrounding conditions. Nothing is more easy than to tame an animal, and few things more difficult than to get it to breed freely under confinement, even when the male and female unite." (Origin of Species.) This fact then also invites a comparison of analytical data from these tissues in their functional and non-functional states. The further fact that the *time relations* of ovulation, and of other activities of the reproductive organs, are easily ascertained in birds—much more easily than in either mammals or reptiles—is an additional and valuable asset in the prosecution of such studies.

The present studies deal with two general types of tissue:

(1) The follicular membrane, the immediate envelope of the growing ovum. This membrane, from ovarian ova of the fowl, was obtained at various stages of its activity, two stages of which have been shown—in the first of this series of studies (3)—to contrast as the figures 1: 25.8 in the rate at which the constituents of yolk pass through this membrane.

(2) The oviduct of the fowl, which was always divided for analysis into two of its functionally differentiated parts; (a) the lower (posterior) shell secreting gland or uterus; and (b) the upper (anterior) albumen secreting portion. The functioning glands (oviducts containing eggs) are contrasted with oviducts free from the presence of eggs (i.e., without the efficient stimulus to the secretory activity of these glands) for longer and shorter periods.

Our analyses of the follicular membranes were made in 1910–1911. The results of these analyses give some support to the view that a *change* in rate of activity, in the direction of greater activity of the tissue, is accompanied by an increased phosphatide content of the tissue; but, after the change in activity is effected, the percentage of phosphatides is little, if at all, higher than in the period which preceded the change. Our evidence on these points would be much strengthened if the per cent of solids in this tissue had proved to be larger, or if the preparation of larger samples had not been discouraged by the excessive tediousness of the process of freeing and cleaning these minute membranes.

The phosphorus values obtained from the albumen secreting portion of the oviduct clearly show that the resting gland (i.e., one that has not functioned for three or four days, and will not again function for a similar period), contains a higher percentage of alcohol-ether soluble phosphorus than does the same gland a few hours before, or a few hours after, the actual discharge of albumen. The alcohol-ether insoluble phosphorus also seems, in a much less pronounced way, to follow the same rule. The several facts obtained on the composition of the various tissues studied, and on the relation of rest and activity to variations in composition, will later be considered in detail.

METHODS OF PREPARATION AND ANALYSIS

Analysis. The methods of analysis used with the *follicular membranes* are those fully described[2] by Koch (4), and are the same as were used and briefly described in the several previous papers of this series of studies. This includes a four-hour extraction with alcohol, followed by a one-hour extraction with ether, and this by another twelve-hour alcohol extraction in a Koch apparatus. The alcohol-soluble, water-insoluble phosphorus was determined by the Neumann-Pemberton method and in all of the tabulated data was calculated (no. cc. of $\frac{N}{2}$ NaOH used x 0.6742 mgm.; then this figure x 25.75) as lecithin (phosphatides). Other phosphorus than lipoid phosphorus forms a part of the figures for ash.

The analysis of the *oviducal tissues* was shortened (extraction same as before) to estimations of moisture, alcohol-soluble and alcohol-insoluble fractions, and to phosphorus determinations on the whole of each of these two fractions. The phosphorus of the oviduct was calculated as P_2O_5.

Preparation. The follicular membranes, of the several sizes, were obtained from the ovaries of laying hens. The ovaries were taken immediately from the just-killed hens. The dimensions given for the membranes are those of the diameters of the ovarian ova which they surrounded.

The preparation of the four samples under *a* and *b* was an extraordinarily tedious task. The second sample under *a* for example required the use of 1020 follicles. The eggs of appropriate size were stripped of the *thecae* with fine forceps; the remaining follicular membrane was then slit open, over about one-half of its circumference with fine scissors, and all traces of yolk quickly washed from the interior with distilled water. The immersed membrane in water tends to retain or re-assume its round shape, and after a few light rapid strokes with a smooth blunt instrument it becomes perfectly transparent and clean. It is then dried to approximately normal weight on filter paper. Unfortunately these membranes proved to have a lower per cent of solids than was expected; and since the labor of obtaining the clean membranes was excessive, the size of many or most of these samples proved to be smaller than is desirable. The figures obtained from the smallest samples are probably not wholly reliable even for the first two (alcohol-soluble and alcohol-insoluble) fractions obtained.

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[2] The sulphur determinations are omitted in all our analyses.

Oviducts were obtained in varying stages of rest and activity. Each complete oviduct (less the vaginal portion) was divided at the line of the isthmus into the upper albumen secreting portion (plus the funnel) and the lower shell gland (plus the isthmus, which secretes the shell membrane). Care was taken not to include in the samples of shell gland any of the fat which often overlies the vagina and sometimes extends upon the shell gland. The oviducts were taken fresh and warm from hens whose eggs had been trap-nested. The fresh samples were weighed, thoroughly crushed in a porcelain crucible and preserved for two to four weeks before analysis in large quantities of 95 per cent alcohol.

THE COMPOSITION OF THE FOLLICULAR MEMBRANE

The two analyses under *a* of table 1 are of membranes from the slow-growing ova of 3 to 4.5 and 3 to 5.5 mm. diameter. The ova enveloped by these membranes contain only white yolk, and this yolk, as noted above, passes through the membrane at about one twenty-fifth of the rate at which yolk later passes through these membranes (3). The data from these two analyses indicate that in this young and relatively inactive stage the membrane contains less phosphorus than in stage *b*, the stage in which the shift to a greatly increased transfer of yolk material occurs. The amount of phosphorus in these smallest membranes is, however, nearly equivalent to the phosphorus content of the much larger membranes *after* the period of increased activity is established (d and dd).

The two analyses (b) of membranes taken closest to, and including, the period of an enormous increase in the passage of yolk materials through the membrane differentiate themselves from all of the other analyses in showing extremely high phosphatide values. One of the two samples yielded a very high alcohol-soluble fraction (27.46), but the other (15.98) is not above the average in this respect. It seems probable that these two samples which yielded the highest phosphorus values contained the smallest amounts of neutral fat. The data certainly indicate that the membranes at this the period of *change* in degree of activity contained least neutral fat in proportion to phosphorus soluble in alcohol and insoluble in water. These analyses therefore indicate that at the period of a *change* in the activity of these

TABLE 1

*The composition of the follicular membrane of the fowl at various stages of its growth and activity**

	NATURE OF MEMBRANE	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS							H_2O
			Alcohol soluble	Alcohol insoluble	Phosphatides† (P as lecithin)	Neutral fat	Protein	Organic extractives‡	Ash‡	
a	3–4.5 mm. follicular membranes (432)....	1.489	17.71	82.29	9.77	6.10	74.66	4.92	4.55	85.21?
	3–5.5 mm. follicular membranes (1020)...	2.188	14.15	85.85	5.13	7.06	76.35	7.54	1.92	84.05?
b	5–6 mm. follicular membranes (223)......	1.624	15.98	84.02	14.00	traces	76.86	4.18	5.01	85.74?
	4–6.5 mm. follicular membranes (no. ?)..	1–2g.?	27.46	72.54	23.10	traces	72.54	2.58	2.58§	
c	5–13 mm. follicular membranes (316).....	2.236	13.72	86.28	3.65‖	7.29	78.42	8.36	2.29	84.18?
d	15–34 mm. follicular membranes (25).....	3.645	14.75	85.25	13.10		77.45	7.94	1.51	87.20?
	18–34 mm. follicular membranes (14).....	2.880	18.02	81.98	8.55	7.05	76.12	4.39	3.88	88.06?
dd	Inner capsules (12) of largest ova; outer layer removed......................	3.110	19.36	80.65	10.27	6.47	75.95	3.59	3.72	87.88?
e	Entire follicles (5) of liberated ova......	1.415	36.18	63.82	12.99	19.93	57.88	3.94	5.26	80.50?

* We are indebted to Miss A. A. Spohn for assistance with this series of analyses.

† The alcohol-ether soluble, water insoluble phosphorus is alone represented here, and is calculated (P x 25.75) as lecithin.

‡ The smallest samples do not permit accurate determinations of extractives and ash; the figures are too high in most cases. Only the data from the larger samples are approximately correct.

§ Organic extractives and ash were here determined on the alcohol-soluble substance only; the *protein* value is therefore too high; the value is that of the entire alcohol-insoluble fraction.

‖ The lecithins here were incompletely precipitated; the figure for phosphorus is therefore too low and the figure for fat is correspondingly too high.

membranes (to a much higher activity), the phosphatide content of the tissue was increased, and the neutral fat content was diminished.

These data are of further interest in connection with the Overton theory of the permeability of membranes. They suggest that among the lipoids the phospholipins play the chief part in a greatly increased permeability of membranes. The fact of an enormous increase in the rate of transference of substances through this membrane at this period (5 to 6 mm.) is firmly established (3); at this point this membrane increases by about twenty-five times its previous rate of passage (or secretion?) of yolk constituents to the ovum. At this same period the ratio of phosphatides to neutral fat is by far the most extreme found in the entire series of analyses. It may be, of course, that in this period of the growth and activity of this tissue an unusual percentage of alcohol-ether-soluble and water insoluble phosphorus exists in molecules much smaller than that of lecithin, and but little in the form of lecithin. This point has not been investigated. The data indicate, however, that the amount of phosphorus having the solubilities just noted, in relation to neutral fat, is much higher than that usually found in other stages of the existence and activity of this tissue.[3]

That the unusually high proportion of phosphatides of analyses *a* to *d* are not to be accounted for by contamination of the membranous tissue with yolk is shown by the fact (5) that in the egg yolk of the fowl the ratio of phosphatides to fats (analyses and calculation the same) is 11: 25 (yellow yolk), and 11: 23 (white yolk). The substance that passes through all these membranes is therefore much richer in neutral fat than in lecithin; but the membrane through which these substances pass, at one important stage at least, contains these substances in wholly reversed proportions.

The membranes composing analysis "dd" are not wholly comparable with the preceding membranes, since only the theca externa, bearing the large blood vessels, was stripped from these nearly mature follicles; leaving here both the theca interna and follicular membrane. The above-mentioned ratio of phosphatides to fat obtains also in this tissue.

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[3] In analysis *c* some membranes from the period of *changing* permeability (5 to 6.5 mm.) were included, and the values found in this analysis should be intermediates of those for b and d. Apparently they are not. The incomplete precipitation of lipoid phosphorus, and the unknown proportions of smaller and larger membranes in the sample, make the value of the analysis somewhat uncertain.

This relation does not persist, however, in the (entire) follicles or capsules which have completed and entirely passed their functional period (analysis e). In this stage phosphatides are to the fats as about 13:20. This is, of course, a degenerating tissue, and on this basis its high fat content is to be expected. If, however, there exists in birds a tissue comparable with the *corpus luteum* of mammals, an improbable supposition, then that tissue should be included in this material, the degenerating follicle. It may be noted that the amounts of water, fat and protein in this tissue depart from the corresponding amounts in all of the preceding tissues, and in the direction of the amounts characteristically found in mammalian luteal tissue.

Several of the analyses were made on smaller amounts of tissue than is desirable, because as already noted, the preparation of the smaller membranes is an extremely tedious task and requires the sacrifice of many animals. The figures obtained for ash and organic extractives from the smallest of these samples are probably too high. Even in the more adequate samples, however, the percentage of water-soluble organic substance is notably high.

The moisture figures on the series of membranes is of interest. Although considerable amounts of connective tissues are present in all these membranes the water content ranges from 80.5 per cent to 88.0 per cent. We do not believe that these samples as prepared and weighed departed at all widely from their normal state, though the possibility for increase or decrease was present in all cases. In the table the accuracy of all moisture figures is questioned.

THE COMPOSITION OF THE OVIDUCAL TISSUES

The shell gland. In table 2 the oviducal tissues are arranged in order of their activity; or better, the shell glands are arranged in the order of imminence of, or readiness for, their period of secretory activity. The glands that would soonest have come into full activity are placed in the top rows of the table.

Noting first the phosphorus values it will be seen that the three oviducts (122, 120, 126) which were ready for their highest activity do not consistently differ in the amount of alcohol-ether soluble phosphorus from the inactive glands (124, 130, 128). On the other hand, the alcohol-insoluble phosphorus seems to be present in somewhat greater amount in the active than in the inactive glands. The total of alcohol-ether soluble substance is appreciably less in the active than in the inactive glands.

TABLE 2

Analyses of functioning and non-functioning shell glands and upper (albumen-secreting) oviducts of the fowl

ANALYSIS	TISSUE	POSITION OF EGG	WEIGHT	WATER	PER CENT MOIST WEIGHT			PER CENT DRY WEIGHT				
					Alcohol soluble	Alcohol insoluble	P_2O_5	Alcohol soluble	Alcohol insoluble	Total P_2O_5	Alcohol soluble P_2O_5	Alcohol insoluble P_2O_5
122	Shell gland	With shell membrane; entering shell gland, (funnel also gripping ovarian egg)	13.92	83.56	3.62	12.92	0.39	21.87	78.13	2.35	3.31	2.08
123	Upper oviduct		30.70	77.72	4.04	18.23	0.20	18.14	81.86	0.91	0.77	0.95
120	Shell gland	With membrane; entering shell gland	17.76	83.88	3.97	12.15	0.32	24.64	75.36	1.97	2.51	1.79
121	Upper oviduct		27.45	79.94	4.26	15.81	0.12	21.23	78.77	0.61	0.88	0.53
126	Shell gland	With shell membrane; 18 mm. above shell gland	11.40	83.81	3.88	12.30	0.44	23.99	76.01	2.74	4.42	2.21
127	Upper oviduct		20.06	77.45	4.59	17.96		20.34	79.66			1.26
124	Shell gland	None: egg laid same day.*	16.60	83.59	4.53	11.88	0.25	27.61	72.39	1.53	1.68	1.48
125	Upper oviduct		27.37	74.33	3.75	21.92	0.28	16.62	85.38	1.09	2.72	0.81
130	Shell gland	None: last egg 3 days earlier†	6.29	80.86	4.68	14.46	0.46	24.45	75.55	2.41	4.71	1.64
131	Upper oviduct		7.81	76.35	5.11	18.54	0.55	21.60	78.40	2.34	4.79	1.66
128	Shell gland	None; last egg 4 days earlier‡.	13.27	81.33	5.75	12.90	0.40	30.82	69.18	2.16	3.01	1.78
129	Upper oviduct		20.42	77.18	5.76	17.06	0.39	25.25	74.75	1.73	3.58	1.10

* An egg in ovary ready to ovulate; would have entered oviduct within a few hours.

† Largest egg in ovary = 17 x 13 mm.; would probably have entered oviduct about three or four days later.

‡ Largest egg in ovary = 14 x 13 mm.; would probably have entered oviduct about four days later.

It is of interest to note that the moisture values obtained from the four shell glands which were ready to function, or had just ceased to function (124), are high and quite nearly equivalent, ranging from 83.46 per cent to 83.88 per cent; while the quite inactive glands gave notably lower values (80.86 and 81.33). It seems not improbable that this incidence of low moisture and tissue inactivity is a fact of importance. The analyses of upper oviducts also show, in the main, a similar association of lower moisture values in the more inactive state of the tissues.[4]

The upper oviduct. The alcohol-ether soluble phosphorus of the active albumen glands (which had ceased their active secretion of albumen only a few hours previous to the taking of the sample and would, within a few hours, again have become active) is small in amount; it is perceptibly increased in those glands which had been inactive, and would certainly have remained inactive, for longer periods. The total phosphorus of the upper oviduct is usually considerably smaller than the percentage found in the shell gland, and this situation is, no doubt, connected with the fact that the secretion product of the upper oviduct—the egg albumen—contains only traces of phosphorus, while in the egg-shell this element is abundant. The alcohol-ether insoluble phosphorus of the upper oviduct apparently does not vary consistently in this series. Probably the total alcohol-ether soluble substance is increased in the inactive state of the glands.

The moisture determinations on these albumen secreting glands offer some evidence that the less active glands contain least water, a situation to which reference was made above.

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[4] Undoubtedly a somewhat higher percentage of blood is present in the active than in the inactive glands, and fowls' blood contains about 86 per cent of water. But in order to raise the water content of the inactive shell gland no. 128 to the average value found for the active glands the former would have to receive an additional quantity of blood equal to its total inactive weight—and this without the addition of any new tissue with less than 86 per cent water. This demonstrates that the higher water values of the active glands cannot be attributed to increased vascularity alone.

On the other hand, it is not improbable that the differences observed in the water content of the active and inactive *upper oviducts* is ascribable to an influx of blood to the active gland. The differences are here smaller in amount, and, owing to the low water content of this tissue, each added gram of blood would here much more greatly affect the percentage of water in the analyzed tissue.

The very small size of the oviduct that furnished analyses 130 and 131 is only partly to be accounted for by shrinkage of tissue under inactivity. The hen from which this oviduct was taken was much smaller than the other hens (all mongrels) killed for this purpose.

Comparing the chemical composition of the upper and lower parts of the oviduct one first notes that the shell gland always has a considerably higher water content than that of the upper oviduct with which it was connected. This brings to light the interesting fact that that part of the oviduct whose secretion is about 90 per cent water, is itself relatively water poor; while the other part, whose secretion is more than 95 per cent solids,[5] is relatively water rich.

Perhaps the question should be asked, Is an albumen secreting gland, or a shell gland, really active (or predominatingly active) only when it is actually discharging its secretion? Are such of these glands as are called upon during a few hours each day, in fowls laying daily, really quiescent during the intervals between discharges of secretion? May not such intervals be the truly active, reconstructive, or preparatory periods? To these questions there is at present no definite answer. There can be no question, however, that the first eight analyses of table 2 represent more active tissues than do the last four analyses of the table. Oviducts completely free from eggs for three or four days, with no possibility of discharging a secretion during the succeeding three or four days, are unquestionably less active glands than those containing eggs and actually engaged, daily or almost daily, in the copious and rapid delivery of their secretory products.

SUMMARY

The membranes (membrana follicularis) which immediately surround the growing oöcytes (of 3 to 34 mm.) of the fowl were subdivided for analysis into (size) groups. Through the membranes (3 to 5.5 mm.) of group *a* yolk constituents are passed extremely slowly to the egg; group *b* includes those membranes (5 to 6.5 mm.) which suddenly, in a day, *change* the former rate of passage of yolk constituents from the blood to the yolk, to a rate about twenty-five times greater; group *d* includes membranes of the largest size (15 to 34 mm.); through these the constituents of yolk are passed or secreted at the rapid, but changeless, rate.

The phosphatides of all three groups of membranes probably exist in amounts relatively large in proportion to the neutral fats.

The greatest disproportion of phosphatides to neutral fats, and by far the largest amounts of phosphatides, apparently are found in group *b*; it is at this stage of the growth or history of the follicular membrane that there occurs a most striking *change* in its permeability. Neither the disproportion of phosphatide to neutral fat, not the high phosphatide value persists under the new rate of permeability, i.e., *after* this new rate is established.

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[5] One statement puts the moisture of the shell at about 1 per cent. In the state in which it is found in the uterus or shell gland the moisture of the shell is certainly considerably higher.

The results lend some support to the view that *changes*, at least a change toward greatly increased activity, in the physiological activity of a tissue is accompanied by an increase in the phosphatides of the tissue. Similarly, the results supply one case in which a change in the permeability of a membrane, toward increased permeability, is apparently accompanied by a perceptible increase in the phosphatide content of the membrane. According to one's classification of the follicular membrane as an active secretory, assimilative tissue, or as a membrane—a more or less passive filter for yolk constituents—the results apply to the one or the other of the above-mentioned problems. Admittedly, however, our results on this topic would be more conclusive if based on larger samples.

Active and relatively inactive oviducts, divided into shell glands and albumen secreting glands, of the fowl were analyzed, and their chemical differences noted.

The alcohol-ether soluble phosphorus of the shell gland (uterus) does not differ consistently in the active and inactive glands. Probably the alcohol-ether insoluble phosphorus is higher in the active glands. The total alcohol-ether soluble substance is greater in the inactive glands. The amount of moisture is plainly and significantly greater in the active glands.

In the albumen secreting glands the alcohol-ether soluble phosphorus is much increased under inactivity. It seems not at all possible to ascribe this increase to tissue shrinkage (or relatively greater loss of other constituents) under inactivity; the absolute amounts of phosphorus are increased, and this in a tissue whose inactivity is beyond question. This result for this gland is in contradiction to the results from the follicular membranes, unless most of the phosphorus of the inactive albumen gland is water soluble; and unless most of that of the active gland is water insoluble. This point remains undetermined. The percentage of water is somewhat larger in the active than in the inactive albumen secreting glands.

The shell glands secrete a substance containing very little water; the upper oviducts secrete a substance very rich in water; but the shell glands, in both the active and inactive condition, contain 4 to 9 per cent more water than do the albumen secreting glands.

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A STUDY OF THE CONDITIONS INVOLVED IN THE ACCUMULATION OF DISSOLVED SUBSTANCES IN THE BLOOD

I. The Relation of Acidity to the Retention of Sugar and of Urea by the Colloids of the Blood and the Kidney

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A considerable number of special studies has been made concerning the conditions involved in the excretion of water by the kidney. It is evident that several factors enter into the process. It is necessary for "free" water (i.e., water not in colloid combination) to be present in the blood, and it is necessary for the kidney to be in condition to excrete it. Under various conditions the colloids of the blood may have more affinity for the water than the kidney tissue, whereas under other conditions, the colloids have less power to hold the water and some of it is set free. At times the kidney tissue may have abnormal retention powers. Fischer's (1) application of the fact that hydrophilic colloids retain more water, the more acid they are, and that the water content is to a certain extent a function of the total acid content (modified, in the case of different acids, by the nature of the anion radical), serves as a simple description of the various kidney states. A more "acid" kidney will tend to retain more water than one in a less acid condition. Excretion will then be reduced. Do similar conditions affect the diffusion of urea, uric acid, creatinine, glucose, chlorides, or other dissolved substances?

PART I

In a previous paper (2) one of us showed that phenolsulphonephthalein is retained by the hydrophilic colloids of blood serum in proportion to the acid content of the serum.

TABLE 1

SOLUTION	$\frac{N}{10}$.HCl ADDED TO SOLUTION	DEPTHS OF COLUMNS OF LIQUIDS TO GIVE SAME COLOR
	cc.	mm.
3 cc. sheep blood serum, 2 cc. 0.8 per cent NaCl, 0.3 cc. 0.6 per cent aqueous solution phenolsulphonephthalein		21
Control: Same, but with 3 cc. 0.8 per cent NaCl instead of the blood serum		17
3 cc. sheep blood serum, 1 cc. 0.8 per cent NaCl, 0.3 cc. 0.6 per cent aqueous solution phenolsulphonephthalein	1	47
Control: Same, but with 3 cc. 0.8 per cent NaCl instead of the blood serum	1	17
3 cc. sheep blood serum, 0.3 cc. 0.6 per cent aqueous solution phenolsulphonephthalein	2	90
Control: Same, but with 3 cc. 0.8 per cent NaCl instead of the blood serum	2	17

In those experiments, the results of which are given in table 1, 3 cc. of each solution, after a thorough mixing, were placed in parlodion sacs prepared after the method of Levy, Rowntree, and Marriott (3). The sacs were lowered into an 0.8 per cent sodium chloride solution until the level of the surrounding solution was the same as that in the sac. Diffusion was allowed to proceed for fifteen minutes, and the sac was then removed and rinsed off with distilled water. The salt solution with the rinsing water was made alkaline by the addition of 10 cc. of 10 per cent sodium hydroxide, and distilled water was added until the volume was 100 cc. The varying shades of pink showed that different amounts of phenolsulphonephthalein had diffused through the walls of the tubes, making a quantitative determination of the dye possible by means of the Duboscq colorimeter. The table shows that as the acid content of a lyophilic colloid (the blood serum) is increased, there is a corresponding retention of the dye, as shown by the lessened diffusion.

Blood serum, however, does not show this behavior toward all dissolved substances. The first group of experiments below, show that in the case of urea and glucose, the blood serum does not retain these substances.

In the following experiments, blood serum with known amounts of acid and of glucose was placed in parlodion sacs, and the contents allowed to diffuse into an 0.8 per cent sodium chloride solution. The technique is described above.

The following protocol will serve to show the results. Three cubic centimeters of the solutions were allowed to diffuse for thirty minutes, and after dilution to 100 cc., the amount of sugar was determined by Benedict's method. (Results expressed in terms of cubic centimeters of the 100 cc. used to reduce 25 cc. of Benedict's solution.)

TABLE 2

SOLUTION	$\frac{N}{10}$ HCl ADDED TO SOLUTION	DIFFUSED SOLUTION OF GLUCOSE TO REDUCE 25 CC. OF BENEDICT'S REAGENT
	cc.	*cc.*
10 cc. blood serum, 5 cc. 25 per cent glucose, 2 cc. 0.8 per cent NaCl		25.25 24.55
Same, but with 10 cc. 0.8 per cent NaCl solution instead of serum		23.60
10 cc. blood serum, 5 cc. 25 per cent glucose, 1 cc. 0.8 per cent NaCl	1	24.40
Same, but with 10 cc. 0.8 per cent NaCl solution instead of serum	1	22.20
10 cc. blood serum, 5 cc. 25 per cent glucose	2	24.50
Same, but with 10 cc. 0.8 per cent NaCl solution instead of serum	2	22.20

This experiment indicates that there is no corresponding variation in glucose retention by the colloids of the serum under variations in both total acid content as well as hydrogen ion content.

A similar series of experiments were conducted using urea solutions in the parlodion sacs. The urea which diffused out in thirty minutes was determined by Benedict's method. The results are shown in table 3.

The table shows that variations in acidity were not accompanied by any corresponding change in the urea retention. It is therefore evident that, since with an increased acid content, the serum shows an increased affinity for phenolsulphonephthalein, but does not show a corresponding behavior towards glucose and urea, the colloids of the blood must exert a *selective adsorption* for some substances and not others. The practical significance of this will be taken up in the general discussion of results.

TABLE 3

SOLUTION	$\frac{N}{10}$ HCl ADDED	UREA IN DIFFUSED SOLUTION
	cc.	*per cent*
10 cc. blood serum, 10 cc. 18 per cent urea, 4 cc. 0.8 per cent NaCl		0.366
Same, but with 0.8 per cent NaCl instead of serum. (10 cc.)		0.375
10 cc. blood serum, 10 cc. 18 per cent urea, 2 cc. 0.8 per cent NaCl	2	0.366
Same, but with 0.8 per cent NaCl instead of serum. (10 cc.)	2	0.405
10 cc. blood serum, 10 cc. 18 per cent urea	4	0.366
Same, but with 0.8 per cent NaCl instead of serum. (10 cc.)	4	0.375

PART II.

Under certain pathological conditions, sugar or urea accumulate in the blood. The experiments in Part I indicate that the accumulation cannot be due to retention by the colloids of the serum, at least on a basis of acid content. May this be due to faulty powers of elimination? We must therefore note the behavior of the kidney colloids toward dissolved substances under various conditions.

While some phases of the study of the retention of substances by blood serum can be satisfactorily investigated by means of the parlodion sac diffusion method, it is necessary to use other means in studying the properties of the kidney colloids. For this purpose we have found that the perfusion method described by Sollmann (4) is admirably adapted to our needs. By this procedure we were able to throw some light on several phases of secretion and retention of both water and dissolved substances by the colloids of the kidney. Briefly the technique is as follows:

In anaesthetized rabbits, cannulas are tied in the renal artery and vein, and in the ureter. The apparatus, as described by Sollmann, is arranged for the perfusion, under uniform pressure, of salt solutions through the kidney. After washing out all the blood with either 0.9 per cent NaCl solution, or 0.9 per cent NaCl and 0.2 per cent to 0.5 per cent glycocol, both solutions being made alkaline (pH = 7.3–7.6), the kidney was removed from the body, mounted on a glass plate in a holder, and evaporation prevented by a covering of cotton, which was wet with 0.9 per cent NaCl made alkaline to pH = 7.8–8.0 with NaOH.

Solutions were then prepared containing 0.9 per cent NaCl and 0.5 per cent urea, and the hydrogen ion concentration was varied with traces of hydrochloric or phosphoric acid, or sodium hydroxide. We then had solutions of practically the same composition, but with different hydrogen ion concentrations. The concentrations used in these experiments were pH = 6.6, 6.8, 6.9, 7.0, 7.2, 7.3, 7.4, 7.5, 7.6, 7.8, 7.9, and 8.0. It will be noticed this range, extending from the acid pH = 6.6 to the alkaline pH = 8.0, covers a series which is well within physiological limits. The variations between the solutions were extremely delicate, and represented gradations that could easily be brought about in the blood of the body during rest, fatigue, feeding, fasting, anaesthesia with chloroform or ether, emotion, or during disease. Besides these solutions, others were used in which the concentrations of the constituents were varied.

The procedure used for any one kidney was to pass the solutions—50 to 150 cc. from an arterial reservoir—into the artery, and collect the vein fluid and the ureter fluid. After passing one solution through, a second, third, or fourth solution followed, the vein and ureter fluids being collected separately. Thus we were able to start with an alkaline solution, and follow it with ones more acid, or use the reverse order. Oxygenation was accomplished by allowing air to bubble through the solution in the arterial reservoir. In each case the hydrogen ion concentration of the artery solution was taken from a specimen drawn from a valve, just before it entered the renal artery. The method used was that of Levy, Rowntree, and Marriott (3). The determinations in all cases were made immediately after collection of the liquid. The experiments covered periods of from fifteen minutes to two hours, varying with the number of solutions passed through. In each case the venous solution was more acid than that of the artery, and the ureter fluid more acid than that of the vein, showing that acids and CO_2 were being eliminated, having arisen from autolysis and oxidation. The amount of urea was constant in the artery, so that variations in excretion of urea could not be due to the variation of supply.

The results, tabulated below (tables 4, 5, 6, and 7), will be discussed under the following headings:

a. Relation of urea excretion to the acidity of the arterial fluid.

b. Relation of acidity of the arterial fluid to the retention of urea by the kidney colloids.

c. Relation of water excretion to the acidity of the arterial fluid.

TABLE 4

Experiment 4. Solutions passed successively through one kidney

HYDROGEN ION CONCENTRATION OF ARTERY pH	UREA IN ARTERY FLUID	UREA IN VEIN FLUID	UREA IN URETER FLUID	$\frac{V+U}{A}$*
	per cent	*per cent*	*per cent*	
7.3	0.5	0.399	0.288	0.687: 1.0
6.6	0.5	0.405	0.208	0.613: 1.0
7.6	0.5	0.411	0.411	0.822: 1.0

* V = per cent of urea in vein fluid.
U = per cent of urea in ureter fluid.
A = per cent of urea in artery fluid.

TABLE 5

Experiment 5. Solutions passed successively through one kidney

HYDROGEN ION CONCENTRATION OF ARTERY pH	UREA IN ARTERY FLUID	UREA IN VEIN FLUID	UREA IN URETER FLUID	$\frac{V+U}{A}$
	per cent	*per cent*	*per cent*	
7.0	0.5	0.430	0.330	0.76: 1.0
7.2	0.5	0.370	0.440	0.81: 1.0
7.5	0.5	0.462	0.459	0.92: 1.0

TABLE 6

Experiment 6. Solutions passed successively through one kidney

HYDROGEN ION CONCENTRATION OF ARTERY FLUID pH	UREA IN ARTERY FLUID	UREA IN URETER FLUID
	per cent	*per cent*
7.3	0.5	0.348
7.5	0.5	0.438
8.0	0.5	0.471
7.8	0.5	0.462
7.7	0.5	0.422

All solutions listed in the order of their perfusion.

Taking into account the artificial conditions to which the kidney was subjected, the absence of nerve and vasomotor control, the difference between salt solution and blood, the differences in the animals at the time of operation, the disturbance in the oxygen supply, the results were fairly uniform, and warrant the conclusions given in the following pages.

A study of these results will show that the amount of urea excreted is independent, to some extent, of the order in which the arterial solutions were used. Thus in table 4, an alkaline solution was followed by an acid solution, and this again by one more alkaline. In table 5, the series is one of increasing alkalinity, while in table 6 the series becomes more alkaline, and is then replaced by ones more acid. The ureter fluids contained a different amount of urea than the vein, and, as the tables show, the amounts varied with the hydrogen ion content. The results show clearly that the *amount of urea excreted increased as the hydrogen ion concentration of the perfusing fluid decreased.* In other words, the less acid the arterial solution, the more urea was excreted.

The artery fluid in tables 4 and 5 contains 0.5 per cent urea. If the ureter fluids were merely an overflow from the blood, and not a "secretion"—selective, from a quantitative point of view, then we could expect a maximum of 0.5 per cent urea in the vein and 0.5 per cent urea in the ureter fluid. Their sum would be 1.0 per cent, and would equal twice that of the artery. The experiment shows that the sum falls short of this, that neither the ureter fluid nor the vein fluid reaches the strength of the artery fluid, and that furthermore, their combined strength falls short of the calculated amount. It is evident that some of the urea is retained by the kidney, and a glance at the tables shows that *the more acid the solution, the more urea is retained.* As the solution approaches pH = 7.6 or the normal H ion concentration of the rabbits' blood, less urea is retained. We would therefore conclude that the lack of secretion of urea in an acid condition of the body is due to its retention by the tissues of the kidney type rather than those of the blood serum, as shown in the first part of this paper. This finds interesting confirmation in the work of Long and Hull (6), who note that the kidney, on analysis, contains more urea than some of the other organs. Their animals were anaesthetized, and therefore they were dealing with kidney tissue at pH = 7.0, which is really "acid" for the rabbit. It therefore contained more urea than the other organs whose colloids probably were more of the type of those found in blood serum. We were able to wash out urea by perfusing a 0.9 per cent salt solution through the kidney, if we passed an alkaline urea-free salt solution through an acid kidney. This suggests a physiological reason for the use of alkalies in conditions of urea accumulation.

We have, then, a mechanism for *selective adsorption* and *selective secretion* on a basis of differences in the nature of colloids, so that one type has greater adsorptive powers than another under different hydrogen ion concentrations. We would suggest giving the name *hylophilic* (hylo—"substance," referring to the solute) to the behavior of the kidney colloids towards urea, and *hylophobic* to the relationship of the serum colloids and perhaps some of the other body colloids to urea. In the end, we are dealing with the law of relative solubilities.

The kidney can take up urea while acid, and secrete it while less acid. The kidney can become less acid by the oxidation of acids, so that we would expect excretion to be a process which is accompanied by oxygen absorption by the kidney. The production of carbon dioxide was shown in our experiments, as the vein solution was always more acid than the artery fluid, and the ureter fluid more acid than the vein. However, the hydrogen ion concentration of the ureter fluid in these experiments was less than that of the acid urine of some types of nephritis, and in most cases the vein and ureter fluids were below $pH = 6$ and were therefore alkaline to methyl red. In some experiments, glycocol (0.2–0.5 per cent) was used to absorb the carbon dioxide formed, and the experiment showed that in every case, cloudy swelling of the kidney was long delayed beyond the time that it appeared when salt solutions alone were used. Although glycocol itself made the solution more acid, the hydrogen ion concentration was restored by the addition of sodium hydroxide, so that in the comparison, the glycocol and the salt solution were of the same degree of acidity.

It is significant that during and just after a meal, while there is more material for the production of urea circulating in the body, the blood and urine at the same time are more alkaline, and, if these results are in any way applicable to the living kidney, it would seem that the two factors work hand in hand to bring about a greater excretion of urea. On the other hand, in fasting, the conditions are just reversed, and a diminished urea production accompanies an acid condition of the blood and urine.

The next phase of the problem is, what is the relation between the hydrogen ion concentration of the artery fluid, and the amount of ureter fluid excreted? The experiments (table 6) show that *the lower the hydrogen ion concentration, the greater the secretion of water.* This conclusion, we believe, bears out Fischer's (1) applications of the laws of colloid swelling under acidosis, to urinary secretions.

It will be noted that a very delicate change in the hydrogen ion concentration causes a marked change in the amount of water excreted. When the solution was too acid, as in experiment 4 (table 7), a toxic effect was exerted on the kidney. It showed a typical cloudy swelling—the picture of an acute nephritis, which it was. Protein was present in the vein fluid and in the ureter fluid, although the artery fluid was a salt solution, and contained no albumin.

TABLE 7

A separate kidney was used for each experiment

EXPERIMENT	COMPOSITION OF ARTERY FLUID	HYDROGEN ION CONCENTRATION OF ARTERY FLUID pH	URETER FLUID PER 100 CC. OF VEIN FLUID
			cc.
1	Ringer's solution, phosphoric acid (trace)...	7.0	1.4
	Similar, but with 0.15 per cent urea..........	7.1	1.7
	Similar, with 0.15 per cent urea..............	7.3	4.2
	Similar, with 0.15 per cent urea..............	7.8	4.6
4	0.9 per cent NaCl; 0.5 per cent Glycocol......	7.3	15.8
	0.9 per cent NaCl, 0.2 per cent glycocol; 0.5 per cent urea..........................	7.3	23.22
	Same..................................	6.6	8.05
	Same..................................	7.6	6.28
6	0.9 per cent NaCl..........................	7.3	13.55
	0.9 per cent NaCl; 0.5 per cent urea..........	7.3	21.03
	Same..................................	7.5	27.39
	Same..................................	8.0	33.75
	Same..................................	7.8	25.80
	Same..................................	7.7	15.10

The diuretic effect of urea, as shown in experiments 4 and 6, (table 7), is of considerable interest. The addition of 0.5 per cent urea to the perfusing solution causes an increase from 15.8 cc. of ureter fluid in experiment 4, to 23.32 cc., whereas in experiment 6, there is an increase from 13.55 cc. to 21.03 cc. Sollmann (3) reports that the diuretic effects of urea cannot be reproduced in the excised kidney of the dog and he attributes this to the fact that the kidney has lost its vitality, or that the blood plasma may have a counteracting effect.

The blood of rabbits normally varied in acidity around pH = 7.3–7.6 (i.e., alkaline) but after the anaesthetic it usually rises to a hydrogen ion concentration of pH = 7.0 (cf. Menten and Crile) (5). A solution, then, of pH = 7.0, while really neutral, is relatively acid for the tissues of the rabbit. *A normal salt solution or a solution of urea*, while usually pH = 7.0 or neutral in reaction, *is in reality "acid" to the tissues.* Such a fluid would naturally not cause an increased excretion, and Sollmann's experiment shows this to be true. If, however, as in our experiments, the acidity of this urea solution is decreased, and brought nearer to the normal for the body, a diuretic effect is noted. Chemically, urea acts as a base in the presence of acids, and tends to neutralize them. In the blood or body fluids, then, it would aid in keeping down the hydrogen ion concentration, an action which would explain its diuretic effects.

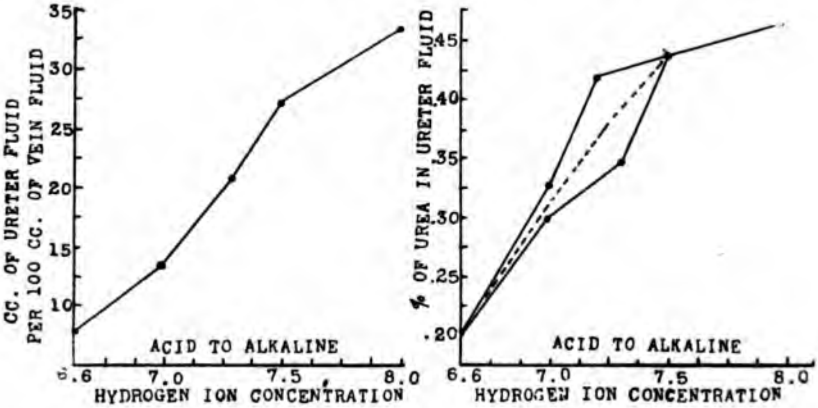

Fig. 1. The first curve shows the relation of the number of cubic centimeters of ureter fluid per 100 cc. of vein fluid to the hydrogen ion concentration of the artery fluid.

The second curve shows the relation of the percent of urea in the ureter fluid to the hydrogen ion concentration of the artery fluid.

The curves represent the mean of several experiments.

Two clinical observations bear out this point of view. After the administration of volatile anaesthetics, the secretion of urine is reduced, and often much delayed. In this condition we have the hydrogen ion concentration of the blood rising to pH = 7.0 or thereabouts, depending on the relative amount of the drug inspired, as Menten and

Crile have shown. Until the blood returns to its alkaline condition, the secretion of urine is lessened. It is further noted that after the intravenous injection of normal salt solution, the effects, while temporary, last for some time, (an hour or so) before the bulk is excreted. When we remember that the normal salt solution is $pH = 7.0$, and is therefore relatively acid, we can account for the delay in immediate excretion, both by the temporary "storage" of water in the serum colloids, and by the effect on the kidney.

While both the water and urea outputs increase with the decrease in acidity (see curve, fig. 1), nevertheless two isolated observations taken are of interest in suggesting that there may be a difference in the water and urea secreting mechanisms. In experiment 4, after the use of a very acid solution ($pH = 6.6$) an alkaline solution was unable to cause a total recovery in the water secreting mechanism, while there was an apparent recovery in the urea secreting mechanism. Furthermore, a kidney which had been kept in the ice-box over night, secreted water the next day, but very little urea. These suggest a subject for more detailed study.

At a given hydrogen ion concentration, only a certain amount of water or urea may be secreted, depending on the available free water or urea. If the supply of either is deficient, the excretion will be less, but the ability to secrete more will still exist. This means that from point of view of hydrogen ion concentration, the ability to excrete water is, in a measure, an index of the ability to excrete urea.

A study of the tables, especially experiments 4 and 6, will show that the previous solution influences, to some extent, the effect of the solution which follows. This is probably due to the extent of hydration of the tissues which a given solution produces, and also to the amount of urea, which is dissolved in the tissue at the given hydrogen ion concentration of the solution. Of course, actual injury to tissue in this method of perfusion results, and the recuperative powers must necessarily be slow, if possible at all.

In continuation of these experiments, it is proposed to test the behavior of the kidney colloids, under similar conditions, towards uric acid, creatinine, and other constituents of the normal and pathological urine.

SUMMARY AND CONCLUSION

1. The colloids of the blood serum show selective *adsorption*, holding phenolsulphonephthalein when the acidity is increased, but acting differently to glucose or urea.

2. In the excised kidney of the rabbit, perfusion of 0.9 per cent NaCl solution containing 0.5 per cent urea shows that under these conditions, and *within the physiological limits of hydrogen ion concentration,*

a. The kidney colloids secrete more urea, the less acid the perfusing solution.

b. The water secretion increases with the decrease in acidity.

c. The amount of urea retained in the kidney colloids increases with the acidity.

d. The urea excretory mechanism, while paralleling the water excretory mechanism in its operation, is quantitatively independent of it.

e. The above changes take place with very minute changes in the hydrogen ion concentration well within physiological limits.

3. The colloids of the body vary in their ability to hold dissolved substances under different degrees of hydrogen ion concentration. This suggests a basis for a mechanism of selective absorption and excretion.

4. The minute amounts of acid or alkali added (somewhere near 0.00035 gram per 100 cc.) to vary the hydrogen ion concentration, are not enough to produce the effects on a basis of osmotic differences, and we believe that we are dealing with a relationship between hydrogen ion concentrations and colloids.

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THE VAGUS NERVES IN PNEUMONIA

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I. INTRODUCTION

In earlier investigations[2] we discovered that the respiratory mechanism is exhausted in pneumonia. The normal stimulus to the bulbar respiratory cells is the carbon dioxide in the blood. To this stimulus the respiratory mechanism in pneumonia reacts imperfectly or not at all.

In pneumonia the observer is struck with the frequency and depth of the breathing. The respirations are often eighty per minute, and, in dogs, the cubic centimeters breathed per minute may rise to eighteen thousand or more. In searching for the cause of this respiratory disorder, attention was directed to the following possible explanations:

1. Such phenomena, occurring in the course of an infection, might be attributed to the action on the respiratory cells of the poison produced by the bacteria of the disease. We have accordingly measured the respiratory reaction in animals in whom the bacteremia of pneumonia was produced, without any disease of the lungs, by injecting the bacillus of Friedländer into the femoral vein.

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[1] Dalton Scholar in the Massachusetts General Hospital.

[2] Earlier papers in these studies of pnemonia are as follows: Arch. of Inter. Med., 1914, xiv, 48; Boston Medical and Surgical Jour., 1914, clxx, 125; *ibid.*, 1915, clxxiii, p. 472; *ibid.*, 1916, clxxiv, pp. 464–466; this Journal, 1914, xxxv, 1–14; Journ. of Exper. Med., 1915, xxii, 123; *ibid.*, 1916, xxiv, 583–603.

The first study cited consisted of measurements of the blood pressure in patients with pneumonia by Newburgh and Minot in the wards of the Massachusetts General Hospital. The remaining studies are of experimental pneumonia. These investigations, as well as the present research, were made in the Laboratory of Comparative Physiology in the Harvard Medical School, and under its direction. The cost of the present research was met in part by a grant from the Proctor Gift, contributed by the Department of Medicine in the Harvard Medical School.

2. With the idea that substances toxic to the nervous system might be formed in the pneumonic lung, the blood was withdrawn from dogs dying of pneumonia and injected into healthy dogs, whose respiratory reaction to carbon dioxide was then measured.

3. The cyanosis so common in pneumonia would suggest that a lack of oxygen might be the cause of the respiratory failure. In order to test this possibility, pneumonic animals were kept in oxygen, the partial pressure of which was many times that of the atmosphere. In other animals, oxygen was passed slowly through capillary areas.

Attempts were made to induce dyspnoea through depriving the animals of oxygen by bleeding. The effect was increased by maintaining the animals in a vertical position.

Further, the removal of oxygen was attempted by depriving the red corpuscles of their binding power by mixing illuminating gas with the inspired air.

Finally, the protection of the respiratory center from exhaustion was sought by giving prolonged artificial respiration.

4. Efforts were made to reproduce the respiratory disorder of pneumonia by placing a dog for many hours in an atmosphere containing from 7 to 12 per cent of carbon dioxide, a quantity sufficient to cause deep respirations at a rate of about fifty per minute.

5. Since the respiratory disorder in pneumonia is manifested both in the lungs and in the respiratory bulbar center, and since these two are connected by the vagus nerves, a causal relation between the dyspnoea and the exhaustion of the respiratory center was sought by section of these nerves. This procedure led us to the discovery that section of the vagus nerves protects the respiratory cells and prevents their exhaustion in pneumonia. Moreover in pneumonic dogs in which both vagus nerves have been cut, the rate of respiration remains normal.

II. GENERAL METHOD

In the experiments mentioned above the condition of the respiratory center was determined by measuring its reaction to carbon dioxide. Haldane has shown that carbon dioxide is the normal stimulus for the discharge of respiratory impulses. When an animal breathes through a closed system of tubes, as in figure 1, the carbon dioxide in the inspired air progressively increases. In normal animals, the increase in the carbon dioxide stimulates the respiratory center and causes a corresponding increase in the volume of the air passing in and out of the chest. Thus, in cats and dogs, when the carbon dioxide rises to 3 per cent, the volume of the air inspired is usually doubled. The condition of the respiratory mechanism is revealed by the volume of air breathed per minute as the carbon dioxide in the inspired air rises from 1 to 5 per cent.

The apparatus for this measurement is shown in figure 1. The tracheal cannula of the animal in which the respiratory reaction was to be measured was joined to a rubber tube placed between two Tissot valves[3] connected in such a way that the animal breathed into a spirometer[4] and out of a bottle connected in its turn with the spirometer, so that the lungs, the spirometer, the bottle, and the connecting tubes formed a closed system. Evidently, by this method the volume of the air passing into and out of the chest was recorded by the spirometer, while the carbon dioxide exhaled by the animal constantly accumulated in the closed system.

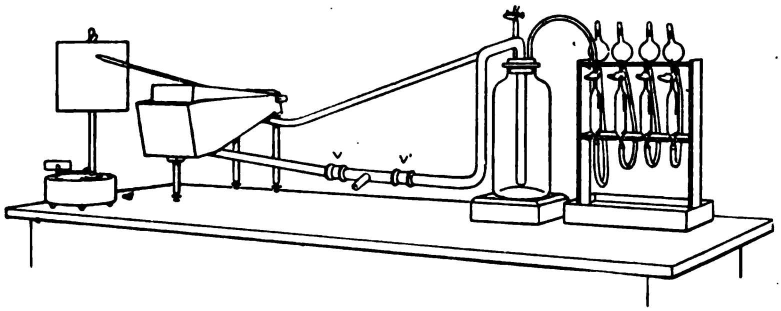

Fig. 1. Apparatus for measuring the respiratory reaction to carbon dioxide. From left to right; the kymograph; the spirometer; the Tissot valves, v,v^1; the bottle; the mercury sample tubes. The electromagnetic signal and the Haldane Apparatus are not shown.

Samples of the air thus enriched with the carbon dioxide were withdrawn at frequent intervals by the mercury tubes shown in figure 1. As each sample was taken, a mark was made on the spirometer record above the time record (the electric time signal is not shown in fig. 1). As the carbon dioxide in the respired air increased, the spirometer curve became deeper until the maximum reaction was reached.[5] The spirometer was empirically graduated by recording the vertical rise of its writing point with each increase of 50 cc. in its contents. When the experiment was finished the empirical graduation scale was used to measure the number of cubic centimeters breathed in the minute nearest the mark at which the air sample was withdrawn. The air in the sample was analyzed for carbon dioxide with a Haldane apparatus and the percentage written against the ventilation (see table 1). Thus, the cubic centimeters breathed per minute as the carbon dioxide rose from the atmospheric level to 3 per cent or more were recorded accurately. When plotted on coördinate paper[6] a curve results, expressing the reaction of the respiratory mechanism to increasing quantities of carbon dioxide. Upon this curve may be read the cubic centimeters breathed per minute at 1, 2, 3, or 4 per cent of carbon dioxide. The data for tables 1 to 4 were obtained in this way.

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[3] Metal flap valves so lightly constructed as not to impede respiration.

[4] This aeroplethysmograph was invented by Prof. J. Gad.

[5] Shown in figure 3.

The organism employed was the *Bacillus* pneumoniae of Friedländer.[7] It was passed through three guinea pigs to increase its virulence to such a degree that 1 cc. of a broth culture injected into the peritoneum killed a guinea pig in twelve hours. Its virulence was kept at this point by occasional passage through additional guinea pigs.

Pneumonia was produced by injecting into the trachea, broth cultures incubated from eighteen to twenty-four hours. The quantity injected varied; we found that 2 cc. per kilo caused death in dogs usually in about thirty hours.

In pneumonic dogs with intact vagi, the fatal issue is usually preceded by a characteristic fall in temperature; this fall was often absent in pneumonic dogs in which the vigi had been cut.

The measurements of pneumonic animals made in this investigation were made only on cats and dogs that afterwards died of the disease, and in whom an autopsy showed the presence of typical consolidation.

All operations that might give pain were performed under ether or morphine or both.[8]

III. PRELIMINARY EXPERIMENTS

The injection of the Bacillus pneumoniae into a vein. In order to separate the possible action of the bacterial poison upon the bulbar cells from the pneumonic process in the lung, a lethal dose of a culture of the Friedländer bacillus was injected into a vein.

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[6] Shown in figure 4.

[7] For the bacteriological examination we are indebted to Dr. C. C Page. See Newburgh, Means and Porter, the Journ. of Exper. Med., 1916, xxiv, 583–603.

[8] For further details regarding method, see Newburgh, Means and Porter, loc. cit.

In the experiment of February 2, 1916, 15 cc. of a broth culture were injected into the femoral vein of a dog at 4 p.m. The rectal temperature rose to 40°C. An hour before death, the temperature had fallen to 38°. The respiratory reaction was measured at six hours and at one hour before death, which occurred thirty hours after inoculation. The second measurement is given in table 1. It was made while the animal was in complete coma, and wholly insensitive to pain. The respiratory reaction is normal. A second experiment also gave a normal result.

TABLE 1

The respiratory reaction to carbon dioxide in a dog in which Bacillus pneumoniae was injected into the femoral vein, causing a fatal bacteremia without injury to the lungs. One hour before death.

PERCENT OF CARBON DIOXIDE IN THE INSPIRED AIR	RESPIRATIONS PER MINUTE	AVERAGE VOLUME OF ONE RESPIRATION	TIDAL AIR PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR
per cent		*cc.*	*cc.*	*per cent*
Room air	16.4	175	2,870	
1.87	17.0	220	3,740	30
3.46	20.4	330	6,730	134
5.16	28.5	460	12,650	340
6.17	54.0	470	25,400	785

These experiments are evidence that the failure of the respiratory center in pneumonia cannot be explained by the bacterial poison in the blood.

The injection of pneumonic blood into healthy dogs. It is conceivable that the pathological process in the lung might develop poisons which, when passed into the blood, would reach the respiratory nerve cells. Evidence against this possibility was obtained by injecting into a healthy dog, previously bled, the blood from dogs about to die from pneumonia. The protocol of this experiment follows:

Experiment on dog 39, March 1, 1916

February 28, 12.30 p.m., dog 37, weight 13.5 kilos, was injected in the trachea with 28 cc. of a broth culture of Bacillus pneumoniae. A second dog, no. 38, weight 17 kilos, received 17 cc. of the culture.

February 29, 7.30 p.m. Dog 37 cannot stand. Temperature 40°C. He is bled 250 cc. from the carotid artery.

8.30 p.m. This blood is introduced into the femoral vein of a normal dog (no. 39), whose respiratory reaction is then tested.

March 1, 1.45 a.m. Dog 38 is in coma. Temperature is falling. He is bled from the carotid artery. The artery is nearly empty. The blood is dark and

thick. 400 cc. of this blood is injected into dog 39 and several hours afterward the respiratory reaction of no. 39 is again measured. It was normal. On room air he breathed 2970 cc. per minute; when the inspired air contained 3 per cent of carbon dioxide, the increase in tidal air was 87 per cent; and at 5 per cent it was 310 per cent.

This experiment gives no support to the hypothesis that the bulbar cells are poisoned through the blood by products arising in the pneumonic lung.

The influence of oxygen and of prolonged artificial respiration. One of the common clinical signs in pneumonia is the early and prolonged cyanosis.

This would suggest that the respiratory disorder could be modified by increasing the available oxygen. Not being certain that the red corpuscles were in a condition to bind their normal quantity of oxygen, it was resolved to place the pneumonic animal in oxygen the partial pressure of which should be great enough to enable large quantities to enter the plasma, thus rendering the respiratory function of the corpuscles more or less superfluous. Pneumonic cats and dogs were placed in a galvanized iron tank provided with a flanged opening. Against this opening, a flanged door, containing a glass window, was firmly bolted. An oxygen cylinder with a reducing valve was connected to a tube leading into the respiration tank and an outflow tube was joined to a safety valve. The animals were surrounded for many hours by oxygen at a pressure of 25 pounds per square inch. The gas within the tank was slowly renewed as it gradually escaped through the outlet valve. A dish of sodium hydrate absorbed some, at least, of the carbon dioxide. As the course of the disease did not seem to be modified by this immersion in oxygen, the experiments were repeated under conditions made more favorable by the continuous removal of the carbon dioxide produced by the animal's respiration. As the apparatus for this purpose may be of interest, a diagram is supplied (fig. 2). A motor drives a pump which forces oxygen through a recording gauge into a galvanized iron tank containing the animal. From the tank the oxygen passes by an outlet valve through sulphuric acid and soda lime into a T tube. One limb of this T conducts the gas to the intake of the pump; the other limb draws from a spirometer fed through a reducing valve from a cylinder containing oxygen under pressure. The outlet valve of the animal tank was almost closed so that the gases passed through the valve at a pressure just sufficient to drive them through the sulphuric acid and soda lime to the intake of the pump.

During this passage the gases were dried and the carbon dioxide removed. Frequent analyses showed that the carbon dioxide in the animal tank was less than 0.5 per cent. The oxygen abstracted by the animal was replaced from the oxygen cylinder by an assistant who allowed gas to pass through the reducing valve at a rate that would keep the spirometer cover from either rising or falling.

In none of the numerous experiments in which the animals were kept for hours in oxygen at a high partial pressure was there any clear evidence that the progress of the disease was checked or the condition of the animal materially improved. It was particularly noticeable that no significant change occurred either in the frequency or the depth of the respiration.

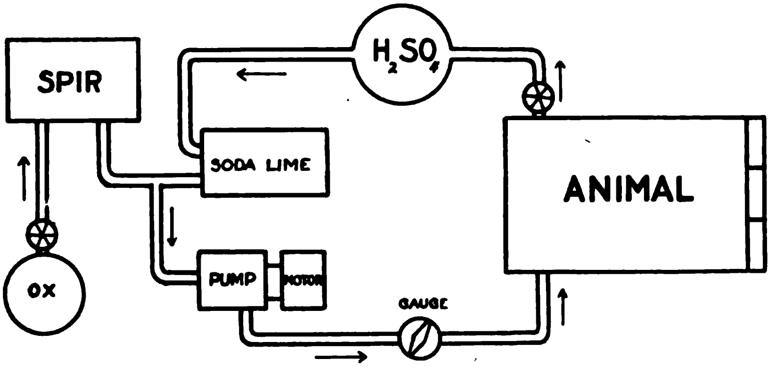

Fig. 2. Apparatus for maintaining an animal under pressure and free from carbon dioxide.

Not having produced a favorable result by immersing the animal in oxygen under pressure, the forlorn hope remained that pure oxygen could be passed through a capillary area so slowly that it might be taken up by the red corpuscles quickly enough to avoid gas embolism. Oxygen was therefore allowed to enter very slowly the peripheral end of the femoral artery, and the femoral vein was exposed to observe the result. The outcome was interesting. A succession of small cylinders of deeply venous blood passed through the vein, each separated from the next by an approximately equal mass of oxygen. No change in the color of the venous blood was observed, even where it was in contact with the gas. After a few minutes, the gas collected in the heart and a rough hissing sound could be distinctly heard with each contraction. Death followed very shortly.

In the attempt to secure evidence that the dyspnoea was due to lack of oxygen, large numbers of red corpuscles were withdrawn by bleeding and the possible anaemia of the bulbar cells was further increased by placing the animals in a vertical position. These experiments failed to produce dyspnoea.

Efforts were also made to produce oxygen hunger by uniting the haemoglobin with carbon monoxide. To this end, illuminating gas was mixed with the inspired air. Dyspnoea did not appear, even when the carbon monoxide was pushed so far that the dogs became comatose.

Since the prolonged dyspnoea in pneumonia must obviously be exhausting, attempts were made to rest the respiratory apparatus by artificial respiration and thus conserve the animal's strength until the crisis might give a favorable turn to the disease. This hope was not realized. The animals with intact vagi appeared to resist the artificial respiration; the frequency of their respiratory discharges did not diminish. Dogs in which section of both vagus nerves caused the irregular spasmodic type of vagal breathing sometimes seemed more comfortable under artificial respiration, and their temperature sometimes fell several degrees, but death commonly followed after the usual interval.

Hyperpnoea from inhalation of carbon dioxide. Trusting that the respiratory center would be exhausted by rapid, deep respirations, extending over many hours, a dog was placed in an air-tight box through which was passed a stream of air containing carbon dioxide and oxygen. The experiment began at 2.30 p.m., February 15, 1916. During nine hours, the dog breathed an atmosphere containing from 7.58 to 12.40 per cent of carbon dioxide. The oxygen varied from 16.6 to 21.8 per cent. The respirations averaged about 50 per minute.

At 11.30 p.m. the dog was taken out of the carbon dioxide box and his respiratory reaction was immediately tested. On breathing room air, the tidal flow was 2800 cc. per minute; on breathing air containing 6.94 per cent carbon dioxide, the ventilation reached the extraordinary figure of 28,200 cc. per minute, an increase of 900 per cent. The respiratory center had not been impaired by the prolonged hyperpnoea.

It will be seen from the preceding account that the number of experiments performed to test each hypothesis was small. Nevertheless, the experiments sufficiently indicated that none of these hypotheses explained the exhaustion of the respiratory apparatus in pneumonia.

Attention was therefore directed toward a fifth possibility, namely, that during the disease, nerve impulses arising in the lung are transmitted along the vagus nerves to the bulbar respiratory cells. These vagal impulses, acting through many hours, might modify the condition of the respiratory cells in such a way that they could no longer react to carbon dioxide. This hypothesis derives weight from the striking difference in the results of injecting a fatal dose of the Bacillus pneumoniae into a vein as compared with those which follow its injection into the lungs. In both cases there is a bacteraemia. When this bacteraemia is associated with inflammation of the lungs, a panting respiration is the most prominent symptom of the disease. When the lungs are not affected, the breathing remains normal until the animal dies. The lungs can reach the bulbar respiratory cells through the blood or through the vagus nerves. The changes in the blood do not produce dyspnoea. We therefore turned to the vagus nerves. The experiments in support of the vagal hypothesis are divided into two groups: first, those in which both vagus nerves were cut in the neck; second, those in which the left vagus was cut in the neck, and the right vagus was cut in the chest after the manner of Pawlow.[9]

IV. THE RESPIRATORY REACTION IN PNEUMONIC DOGS WITH BOTH VAGI CUT IN THE NECK

Table 2 presents the respiratory reaction to carbon dioxide in pneumonic dogs in which both vagus nerves were cut on a level with the upper rings of the trachea. It will be noted that these measurements were made from two and three-quarters hours to fifteen minutes before death. The average reaction in normal dogs on inspiration of air containing 3 per cent of carbon dioxide is an increase of 96 per cent in the tidal air per minute. In the six vagotomized pneumonic dogs, the reaction is 84 per cent. One dog increased 142 per cent. Dog 45 gave a very poor reaction. This was because we were unable to get him to breathe quietly at the beginning of the test. If this experiment be omitted, the remaining five, when the inspired air contained 3 per cent of carbon dioxide, will show an increase of 91 per cent in the tidal air. The reaction in these vagotomized pneumonic dogs was normal. On the other hand, dogs at the same stage of the disease but with vagi intact increased their tidal air by only 46 per cent. Hence, section of the vagus nerves protects the respiratory mechanism from the impairment always observed in this disease when the vagus nerves are intact.

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[9] Pawlow, J. P.: The Work of the Digestive Glands (transl. by W. H. Thompson), Chas. Griffin & Co., 1910. 2nd English Ed. 54.

TABLE 2

Respiratory reaction to carbon dioxide by pneumonic dogs with both vagi cut in the neck during the course of the disease

NUMBER OF DOG	EXPERIMENT	WEIGHT	DATE 1916	HOURS BEFORE DEATH	RECTAL TEMPERATURE	INITIAL VENTILATION PER MINUTE	INITIAL RESPIRATION RATE PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR AS CARBON DIOXIDE IN THE INSPIRED AIR INCREASED FROM 1 TO 3 PER CENT		
								1	2	3
		kilos				*cc.*		*per cent*	*per cent*	*per cent*
44	B	5.5	March 11	¾	35°	2,270	13	14	45	93
45	A	8.0	March 12	½	40°	4,640	18	17	32	45
51	B	4.5	March 14	2	31°	1,500	8	24	57	88
54	A	8.5	March 26	½	40°	3,300	20	18	42	64
56	A	6.5	March.25	2¾	36.5°	3,040	13	11	42	70
57	B	6.5	March 26	¼	39.5°	2,520	21	48	116	142
Average.........		6.6				2,878	16	22	56	84

The reaction to carbon dioxide in the pneumonic vagotomized dog recorded in table 2 is indeed greater than the reaction observed in three vagotomized dogs without pneumonia. The measurements of these normal vagotomized dogs are given in table 3. At 3 per cent of carbon dioxide they increased their tidal air by 68 per cent.

It will be observed in table 2 that the initial ventilation averages 2878 cc. per minute. This is not unusual in dogs, the average weight of which is but 6.6 kilos. Thus in an experiment performed April 13, 1916, a dog wholly normal, except for tracheotomy, had an initial ventilation of 1610 cc. per minute. When the air inspired contained 3

TABLE 3

Respiratory reaction to carbon dioxide in normal dogs with both vagi cut in the neck

NUMBER OF DOG	EXPERIMENT	WEIGHT	DATE 1916	INITIAL VENTILATION PER MINUTE	INITIAL RESPIRATION PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR WHEN THE CARBON DIOXIDE IN THE INSPIRED AIR INCREASED FROM 1 TO 3.5 PER CENT			
						1	2	3	3.5
		kilos		*cc.*		*per cent*	*per cent*	*per cent*	*per cent*
58	A	8.0	March 29	3,182	25	23	44	66	90
66	A	5.5	April 7	1,870	8	20	42	79	104
71	C	4.5	April 14	1,460	12	21	41	60	85
Average..............................				2,171	15	21	42	68	93

per cent of carbon dioxide, his ventilation increased 118 per cent. His weight was 4.5 kilos.

The reader is reminded that the decrease in rate after double vagus section is usually held to be balanced by an increase in depth, so that the total ventilation remains substantially the same. Without making any general statement with regard to this, it may be pointed out that the initial ventilation in two of the three vagotomized but otherwise normal dogs listed in table 3 was greater than the ventilation in the wholly normal dog of April 13.

The reader will also remember that Haldane has conclusively shown that ventilation is not to be measured simply by the tidal air. A difference between the air that passes in and out of the chest and the air that passes in and out of the alveoli must be kept in mind. Deep infrequent breathing may ventilate the alveoli better than frequent shallow breathing, even when the shallow breathing brings more air per minute into the chest. In shallow breathing, a greater portion of the tidal air may be spent on the dead space. In short, it does not follow that a small ventilation in small vagotomized dogs indicates abnormally small tidal movement or is necessarily an evidence of imperfect ventilation.

If the five vagotomized dogs in table 2 whose initial ventilation is small reacted vigorously to carbon dioxide because of their small initial ventilation, a vagotomized dog whose initial ventilation is large should react less vigorously. In our experience this is not the case. On June 13, the respiratory reaction was measured in a vagotomized dog (no. 91) fifty minutes before death from pneumonia. The initial ventilation was 5140 cc. per minute. When the inspired air contained 3 per cent of carbon dioxide, the ventilation was doubled. On June 28, a vagotomized dog (no. 100) was tested just before death from pneumonia. His initial ventilation was 5300 cc. per minute. At 3 per cent of carbon dioxide, the ventilation was 10,000 cc. per minute.

When the tidal air per minute is divided by the weight of the animal in kilos, a ventilation which appears small may be found normal. Thus the average ventilation of the six vagotomized pneumonic dogs in table 2 is 2878 cc. per minute. The dog of June 13 had an initial ventilation 5140 cc. per minute. But the dogs of table 2 weighed as an average 6.6 kilos and the dog of June 13 weighed 12 kilos. The ventilation per minute per kilo in the dogs of table 2 was 437 cc., while in the dog of June 13, which weighed twice as much, it was 442 cc. If this dog of June 13, which weighed 12 kilos and breathed 442 cc. per minute per kilo is compared with the vagotomized pneumonic dog 56 (table 2), who weighed one-half as much but who breathed 468 cc. per minute per kilo, it is at once clear that the small initial ventilation of the vagotomized dogs of table 2 is apparent rather than real.

In our communication on "The Respiratory Mechanism in Pneumonia," we showed at length that the reaction to carbon dioxide was independent of the initial ventilation. It is not necessary to go over this ground again.[10]

It will hardly be denied that a critical examination of table 2 bears out the assertion that double vagotomy prevents the exhaustion of the respiratory center.

V. THE RESPIRATORY REACTION IN PNEUMONIC DOGS WITH ONE VAGUS CUT IN THE CHEST AND THE OTHER VAGUS CUT IN THE NECK

For our purposes, the section of both vagus nerves in the neck has serious disadvantages. If the nerves were cut during the course of the disease, the dogs would sometimes become markedly cyanotic and would then struggle, apparently for oxygen. The breathing in these cases was usually spasmodic and irregular. Such animals are obviously unsuited for testing the respiratory reaction. None such were used in table 2, though one restless dog (no. 45) was accepted with the reservation already noted. Moreover, death often took place before the period at which it would have been expected had not the vagus nerves been cut. As it was important for our results to test the respiratory reaction just before the death from pneumonia, premature death from an intercurrent operation could not be passed over. To avoid this difficulty, we planned to cut the vagus nerves and to allow the animals to recover before they were inoculated. This proved to be impracticable. After double vagotomy in the neck, the dogs could retain neither food nor drink. They all died within three days. It seems that a similar obstacle was encountered by Pawlow,[11] who overcame it by severing the right vagus in the chest, between the cardiac and the pulmonary branches. This point is considerably distal to the origin of the recurrent laryngeal nerve. As the procedure has been highly useful, a description of our technique may not be out of place.

A strong, short-haired dog[12] weighing about 8 kilos is tied upon a well scrubbed board and etherized. When the anaesthesia is sufficiently deep, the mouth is held open by straps passing behind the canine teeth, the tongue is drawn forward and a rubber tube almost the size of the trachea is passed through the glottis down to the bifurcation. At the other end of the tube is a glass Y, one limb of which is left open, to be stopped at will by the finger of the anaesthetist. The third limb is connected with an ether flask through which passes a continuous air current. An additional inhalation of ether can be secured by standing the ether flask in a basin of warm water. The hair is now closely cut over the right upper chest, which is then thoroughly scrubbed with a broad nail brush and hot soapsuds. This region, except the second intercostal space, is then covered with clean towels soaked in corrosive sublimate solution. The instruments are two large, broad retractors, one long and one short dissecting forceps, curved and straight scissors, a right-angled artery hook, a "seeker," six long-handled haemostatic forceps, eight straight and four curved threaded needles, several ligatures, a knife, and a dozen "sponges" of absorbent cotton wrung out of corrosive sublimate solution and placed in a sterile beaker. The instruments were thoroughly boiled before each operation, and were then laid out upon a small movable stand which had been covered with a sterile cloth. The operator, the assistant, and the anaesthetist wore the usual white suits. The operator used a frontal mirror to light the interior of the chest. An incision was made through the skin and the muscles over the second intercostal space from the border of the axilla to the edge of the sternum. The chest was opened in this line and the opening widened with two retractors, held by the assistant. Care must be taken not to press on the superior vena cava. The right vagus nerve lies on the trachea beneath the superior vena cava. The connective tissue is grasped with the long dissecting forceps, the nerve is freed with the seeker, held gently with the forceps, and severed on both sides of that instrument. The removal of a portion of the nerve forestalls any doubt as to its section. The right vagus nerve lies beneath the superior vena cava; the right phrenic nerve lies upon that vein and must on no account be injured. With practice, the vagus operation can be done in a few minutes. When the retractors are withdrawn, the divided pectoralis major muscle is brought together with three or four deep ligatures placed with curved needles. The blades of a forceps are now passed into the chest between two of the deep ligatures and the anaesthetist, by stopping the free end of the Y tube with his finger, causes the lung to expand and drive the air out of the chest. At this moment the forceps is rapidly withdrawn and the skin wound closed with ligatures placed about 1 cm. apart. No dressing is used. Throughout the operation, the dog must be kept anaesthetized by stopping the free end of the Y tube sufficiently often to reproduce the natural respiratory movements of the lung. This intratracheal anaesthesia avoids a tracheotomy, the healing of which is very difficult.

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[10] See The Journal of Experimental Medicine, 1916, xxiv, 583–603.

[11] Pawlow, J. P. loc. cit.

[12] Dogs with distemper should be carefully avoided.

The operation can be warmly recommended. We rarely failed to secure a good recovery within a few days. From three days to a week after the first operation, a small incision was made in the neck over the left vagus nerve, the carotid sheath with the nerve brought to the surface with a curved blunt instrument, and the nerve freed and severed.

Twenty-four hours after the double vagotomy had been completed, the dog ate and drank with comfort. The breathing was vagal in type, but was so easy that the increased depth and the diminished rate might readily escape notice. Indeed, the vagal type could not be made out unless the animal were resting quietly.

TABLE 4

Respiratory reaction to carbon dioxide in pneumonic dogs with one vagus cut in the neck and one cut in the chest previous to the disease

NUMBER OF DOG.	EXPERIMENT	WEIGHT	DATE 1916	HOURS BEFORE DEATH	TEMPERATURE	INITIAL VENTILATION PER MINUTE	INITIAL RESPIRATION PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR AS THE CARBON DIOXIDE IN THE INSPIRED AIR INCREASED FROM 1 TO 3 PER CENT		
								1	2	3
		kilos				cc.		*per cent*	*per cent*	*per cent*
91	C	10.5	June 13	50 min.	102°	5,140	21	20	53	102
100	C	12.0	June 28	2 min.		5,100	12.5	28	57	88
Average.......		11.3				5,120	17	24	55	95

These animals were usually inoculated with pneumonia from three to seven days after the second vagus nerve was cut. The disease ran its usual course except that the characteristic final fall in temperature was frequently absent and the sudden death was not infrequent. Some animals were lost because they died before partial coma had set in, thus preventing the testing of the respiratory reaction. The animal must be quiet throughout this test.[13] Ether is of course impossible. Decerebration cannot be used with double vagotomy because this conjunction produces long, irregular respiratory spasms. It is therefore necessary to wait until the animal is at least partially comatose.

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[13] The test has often been made on men and women. It is quite painless.

In pneumonic dogs with vagi intact the hour of death can be approximately calculated, but in pneumonic dogs with both vagi cut this calculation is much more difficult. The observer who may repeat these experiments will do well to remain continuously with his animals, as we have done, during the thirty or forty hours of the disease.

The respiratory reaction to carbon dioxide in pneumonic dogs with both vagi cut in the manner described above is shown in table 4. The reaction of dog 91, fifty minutes before death, is shown in figures 3 and 4. The protocol of this experiment follows:

Experiment June 2, 1916. Dog No. 91, weight 10.5 kilos

June 2, 11 p.m. Right vagus cut inside the chest.

June 7, 4.10 p.m. Left vagus cut in the neck.

June 12, 4.15 p.m. Bronchus injected with 3 cc. per kilo broth culture of Bacillus pneumoniae.

June 13, 9.15 a.m. Temperature 105°, respiration 18.

June 13, 11 a.m. Temperature 104.8°. Carbon dioxide reaction measured (A).

June 13, 11.30 a.m. Temperature 101.5. Second measurement (B).

June 13, 12.30 p.m. Temperature 101.3. Coma. Third measurement (C) of carbon dioxide reaction.

June 13, 1.20 p.m. Death.

Autopsy. Both vagus nerves severed. Red hepatization of three-fourths of right lower and one-half of right middle lobes.

An examination of table 4 and figures 3 and 4 shows that the respiratory reaction of these vagotomized pneumonic dogs is normal, the average increase in tidal air is 95 per cent, when the inspired air contains 3 per cent of carbon dioxide. In pneumonic dogs with intact vagi, this reaction averaged 46 per cent. In normal dogs, it averaged 96 per cent. The dogs in table 4 had a fairly large initial ventilation, averaging 5120 cc. per minute, or 453 cc. per minute per kilo. The ventilation of five normal dogs averaged 4344 cc. per minute. As in table 2, the dogs with double vagotomy have no dyspnoea in spite of a disease in which dyspnoea is, with intact vagi, the most prominent symptom.

A critical examination of the experiments in table 4 would naturally consider the duration of the disease. The average duration of life in pneumonic dogs with intact vagi, is thirty hours. The six dogs of table 2, in whom both vagi were cut in the neck during the course of the disease, also lived, as an average, thirty hours. Section of both vagi in this manner and at this time does not therefore shorten the duration of the pneumonia. On the other hand, ten dogs in whom, previous to

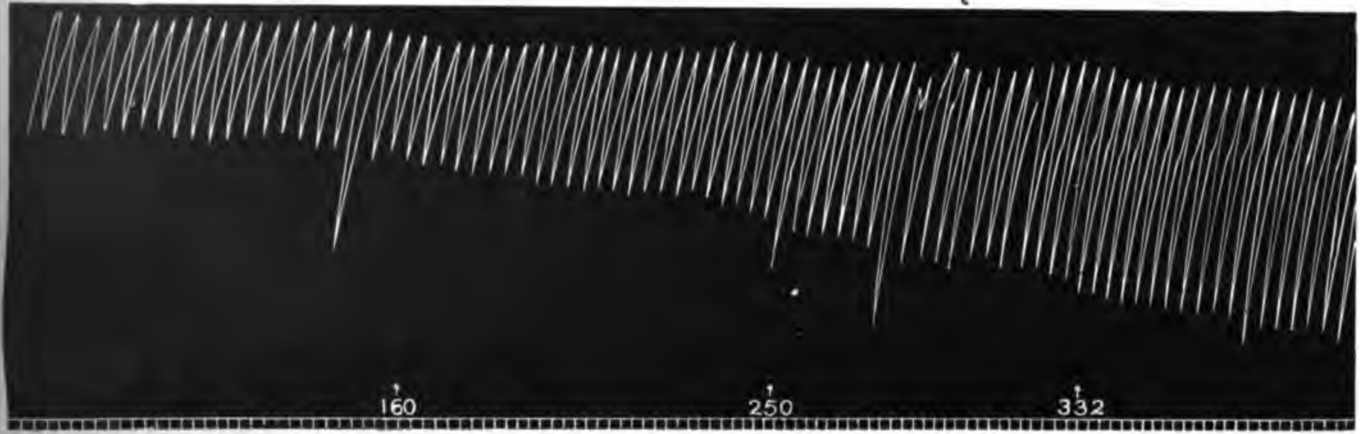

Fig. 3. Normal respiratory reaction of the vagotomized dog, no. 91, June 13, 1916, fifty minutes before death from pneumonia. In room air, this dog breathed 5140 cc. per minute. In air containing 3 per cent of carbon dioxide, the tidal air increased 102 per cent.

inoculation, one vagus was cut in the chest and the other in the neck, lived, as an average, but twenty hours after inoculation. It might, therefore, be said that the dogs in table 4 gave a normal reaction because they were tested ten hours before the pneumonia alone would have killed them. Out of this criticism two questions arise: First, does pneumonia ever kill dogs with intact vagi in twenty hours? Second, if there are such cases what respiratory reaction do they give shortly before death; or, in other words, what is the result of measuring their reaction at the same period in the disease at which the reaction of the dogs in table 4 was measured? These questions are answered by the experiments of November 26 and November 28, 1915. In the first of these experiments, dog no. 6 lived twenty-three hours and his respiratory reaction at 3 per cent of carbon dioxide was 60 per cent measured three hours before death. His temperature was 35°C. This same dog two hours previously gave a normal reaction but his temperature at that time was 38°C. It had probably been higher but since it fell finally to 33° there is some reason to believe that the fatal fall had just begun at the time when this perfect reaction was obtained. The second dog observed on November 28 lived eighteen hours. His respiratory reaction was taken one hour before death when the temperature

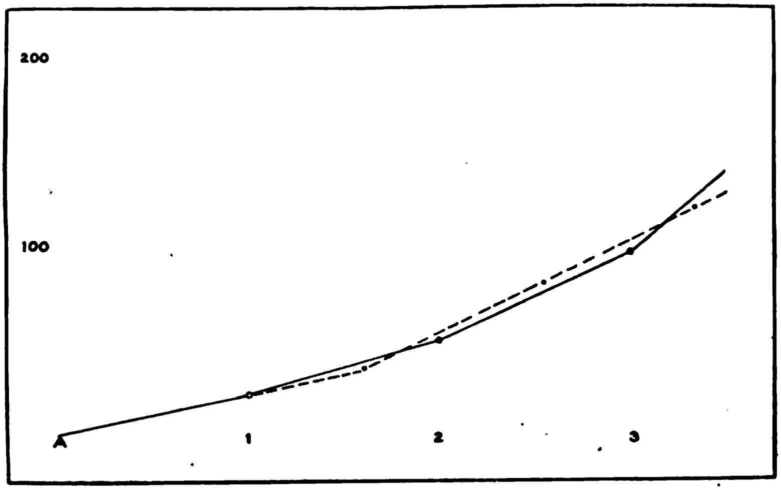

Fig. 4. The broken line is the respiratory reaction of a vagotomized dog (no. 91) fifty minutes before death from pneumonia. The unbroken line is the average reaction of five normal dogs.

was 34° and the usual coma was present. When the carbon dioxide in the inspired air was 3 per cent his tidal air was increased 64 per cent. These facts justify the following statement. Out of five pneumonic dogs with intact vagi, two lived no longer than the dogs in table 4. When the inspired air contained 3 per cent of carbon dioxide these two increased their tidal air by 62 per cent, whereas the dogs in table 4 measured at the same stage of the disease increased 95 per cent.

Additional strength is imparted to table 4 by the history of the third dog of the group of five just mentioned. This animal, no. 14, gave on December 10, 1915, an increase of only 12 per cent in the tidal air when the inspired air contained 3 per cent of carbon dioxide, twenty-four hours after inoculation.

VI. CONCLUSIONS

1. Section of both vagus nerves prevents the exhaustion of the respiratory mechanism in experimental pneumonia.
2. In such animals there is no dyspnoea. The rate of respiration remains practically unchanged throughout the disease.

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STUDIES IN EXPERIMENTAL GLYCOSURIA.

XI. Retention of Dextrose by the Liver and Muscles and the Influence of Acids and Alkalies on the Dextrose Concentration of the Blood

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A considerable amount of evidence has been accumulating in recent years to indicate that the behavior of carbohydrate in the animal body is in part dependent upon the reaction of the tissue fluids. Since conditions such as dyspnoea, etc., which are known to raise the H-ion concentration of the body fluids, cause hyperglycaemia and glycosuria, it was suggested by one of us several years ago that such an increase in the H-ion concentration of the blood supplying the liver cells might stimulate the glycogenolytic process. Direct evidence in favor of this view—and also showing that the opposite change in reaction (increased alkalinity) has a glycogen-forming influence (at least in the turtle liver)—was furnished by Elias (1), who found in rabbits, after administration of dextrose along with acid, that distinctly less glycogen accumulated in the liver and that the blood-sugar rose much more than after dextrose alone. Glycosuria also became established in the former but not in the latter case. Similar results were obtained by this investigator in dogs; thus, the administration of an amount of dextrose which ordinarily caused no glycosuria did cause it when the dextrose was given along with mineral acid, the blood-sugar being also considerably raised in the latter cases. The amounts of acid used in these experiments were such as had previously been shown by Walter to produce distinct changes in the reaction of the blood. Elias and

Kolb (2) subsequently showed that starvation diabetes in dogs is accompanied by acidosis, and that the hyperglycaemia produced by giving a certain amount of dextrose to such animals is more pronounced than that observed after the same amount of dextrose given to normal animals.

Regarding the action of alkali in causing a decrease in the sugar-concentration of the blood, or, in cases of glycosuria, in that of the urine, several researches have recently been recorded. These date from the discovery, announced in 1911 by Pavy and Bywaters (4), that injection of alkali into the portal system inhibits post-mortem glycogenolysis in the liver, and that the glycosuria usually produced by ether and chloroform in cats is abolished by the injection intravenously of about 0.75 grams per kilogram of sodium carbonate (Pavy and Godden (5)). These authors also stated, though they gave no data, that irritation of the vagus nerve in the neck failed to cause glycosuria in cats to which sodium carbonate had previously been given. Murlin and Kramer (6) have discovered that the glycosuria in complete pancreatic diabetes can be caused to disappear by the injection of 300 cc. of a 1 per cent solution of Na_2CO_3, and that meanwhile the blood-sugar does not at least increase, and Underhill (7) has recently shown in normal rabbits that the intravenous injection of 80 to 100 cc. of 0.4 per cent Na_2CO_3 causes a hypoglycaemia lasting for one to one and one-half hours, but capable of being maintained by renewal of the alkaline injection. By administration of alkali (0.5 grams) at an interval of one-half to one hour previous to that of adrenin, this author has also found that the blood-sugar does not rise so high, nor does the increase last for so long a time, as in normal rabbits after a similar injection of adrenin, and the glycosuria is much less.

The undoubted hypoglycaemic effect of alkali administration and the probable hyperglycaemic effect of acid might be explained as due either to an influence on the glycogenolytic process in the liver (and muscles) or to alterations in the rate of sugar utilization of the tissues. To throw light on this question, Murlin and Kramer (6) have observed the behavior of the respiratory quotient following the administration of Na_2CO_3 to normal, and to partially and totally depancreated, dogs. No increase in the respiratory quotient was found to occur in the normal animal, but when the alkali was given intravenously, along with dextrose to a diabetic animal, whose urine had been rendered sugar-free even while on full diet, by previous administration of Na_2CO_3, a distinct rise in the respiratory quotient was observed. A limited capacity to oxidise glucose, in another partially depancreated dog, was found to be increased by giving Na_2CO_3 along with glucose, although Na_2CO_3 alone had no influence. Administration of the carbonate and glucose was also found by these authors to cause a marked rise in the respiratory quotient on the fifth day after total pancreatectomy in a dog.

These results are interpreted as indicating that the alkali stimulates increased utilization of dextrose in the tissues. This might alone be sufficient to account for the decrease in blood-sugar concentration, but there is nothing in the experiments which negatives the view, originally suggested by Pavy's work above referred to, that an equally important cause may be a lessening in the rate of discharge of dextrose from the liver. To investigate this possibility, Kramer and Marker (8) estimated the glycogen in the liver and muscles of a totally depancreated dog that had been caused to retain considerable quantities of dextrose while on a meat diet, by administration of sodium carbonate. Since only traces were found, they conclude that the "retained" dextrose cannot have been stored as glycogen. Evidently recognizing the possible fallacies that are well-known to be incurred in such experiments, the authors performed a second experiment, in which a totally depancreated dog was first of all rendered a-glycogenic by adrenin administration, and was then given meat and carbonate until considerable glucose, which must have been derived from protein, had been retained. On again giving adrenin, it was found that no increase in sugar-excretion occurred, thus indicating that "none of the glucose retained had been stored as glycogen," nor could the dextrose be shown to be "eliminated by way of the saliva or the gastro-intestinal tract." Until further details are available it is impossible to appraise the value of the results of these well-conceived experiments.

The apparent rapidity with which the hypoglycaemic effect of alkali becomes developed (cf. Underhill) suggested to us that it might be possible to decide whether this depends on a depression of the glycogenolytic activity of the liver, by comparison of the sugar-content of the blood flowing into and out of the liver, and likewise whether there was increased utilization (or storage) of dextrose by the muscles by watching the behavior of the sugar in the venous blood of muscles. This has been the general nature of the experiments herein recorded, and in undertaking them we have recognized the fact that it is only when a very considerable retention or secretion of the sugar is occurring that any changes in the comparative amounts of sugar in the inflowing and outflowing blood are likely to be encountered. This objection however applies mainly when the attempt is made to base conclusions on the analysis of one or two samples of blood removed simultaneously from the two vessels. When the samples are removed from the two vessels at frequent intervals, thus permitting curves to be drawn, the method becomes of much greater value, although the differences can never be large. The introduction of methods for rapid sugar estimation in small amounts of blood (2 cc.) has rendered such investigations possible.

Besides the above mentioned investigations concerning the influence of acid and alkali on the behavior of the blood-sugar, we have incidentally collected some data bearing on the cause of the very rapid disappearance of intravenously injected dextrose. A very complete review of the literature bearing on this subject has recently been published by Kleiner (9). The main fact is that, after the injection of large amounts of dextrose, for example about 4 grams per kilo body weight, the injected sugar rapidly disappears from the blood (in ninety minutes in Kleiner's experiments). Although under ordinary conditions some of this excess of dextrose escapes with the urine, it was found by Kleiner still to disappear at about the same rate in animals from which the kidneys had been removed. In Kleiner's experiments conversion into glycogen by the liver could not be held accountable for the disappearance of the dextrose, since a similar disappearance also occurred in animals having no circulation posterior to the level of the diaphragm. It was found that the muscular tissues absorb the excess of sugar possibly by building some of it up into polysaccharides.

There is one serious objection to all of the experiments of the above type, namely, that by the rapid addition of excessively large quantities of dextrose to the blood, conditions are established which can never obtain in even the severest varieties of experimental diabetes. Apart from the disturbance of osmotic relationships between the blood and tissue fluid created by such injections, the excess of dextrose, either because of its own presence or because of decomposition products derived from it (e.g., lactic acid, etc.), may completely throw out of working order those mechanisms which under ordinary circumstances would take care of any physiological excess of dextrose.

Notwithstanding these criticisms, the experiments of Kleiner and others are noteworthy in that they show the glycogenic function of the liver to be of practically no consequence in absorbing the excess of dextrose. This conclusion prompted us in the present investigation to make some observations on the relative sugar-retaining power of the liver and muscles during the continuous injection into the portal vein of large quantities of dextrose.

In the majority of our experiments we have employed for injection quantities of dextrose that were below the normal tolerance limit as defined by Woodyatt, Sansum and Wilder (13). These authors have shown that, in a normal rabbit, dog or man, 0.9 gram glucose per kilo body weight and per hour can be utilized by the organism for an indefinite time without causing glycosuria. When between 0.8 and 2 grams are injected a part of the excess appears in the urine, steadily increasing until a maximum is reached, after which the excreted fraction remains constant (at about one tenth). If more than about 2 grams per kilogram an hour are injected, "a large percentage of all glucose in excess of the 2 grams per kilogram an hour appears in the urine once constant conditions are established."

In the light of these observations we should expect that the curve of blood-sugar concentration during continuous injections of dextrose would gradually rise to a certain level, after which it would remain constant at a height proportional to the rate of injection. With injections below the tolerance limit, the rate of injection at which the curve ceases to rise would indicate the rate at which the tissues are consuming the dextrose, and it is when this point of the curve is reached that the influence of various conditions on sugar utilization by the tissues could be most simply studied. When larger amounts of dextrose are given, the level would represent the balance between sugar loss by the urine *plus* its utilization by the tissues, on the one hand, and injection rate, on the other.

Technique. 2 cc. samples of blood were collected, in the manner previously described (10), from the pancreatico-duodenal vein, the inferior vena cava opposite the liver, and in some experiments also, from the common iliac vein, after ligation of the vessels of the opposite posterior extremity. The samples were collected before, during and following the injection, through a cannula inserted in one of the smaller tributary branches of the portal vein, of the solution whose influence on blood-sugar concentration it was desired to study. To maintain a constant rate of injection—which is absolutely essential in all work designed to study the fate of injected dextrose—either the injection apparatus described in a previous paper or Woodyatt's apparatus (13) was used. Unfortunately, after some of the earlier experiments had been completed, it was found that the air pressure used in the former apparatus was insufficient to ensure a perfectly uniform rate of injection, with the consequence that some of the results are confusing and un-

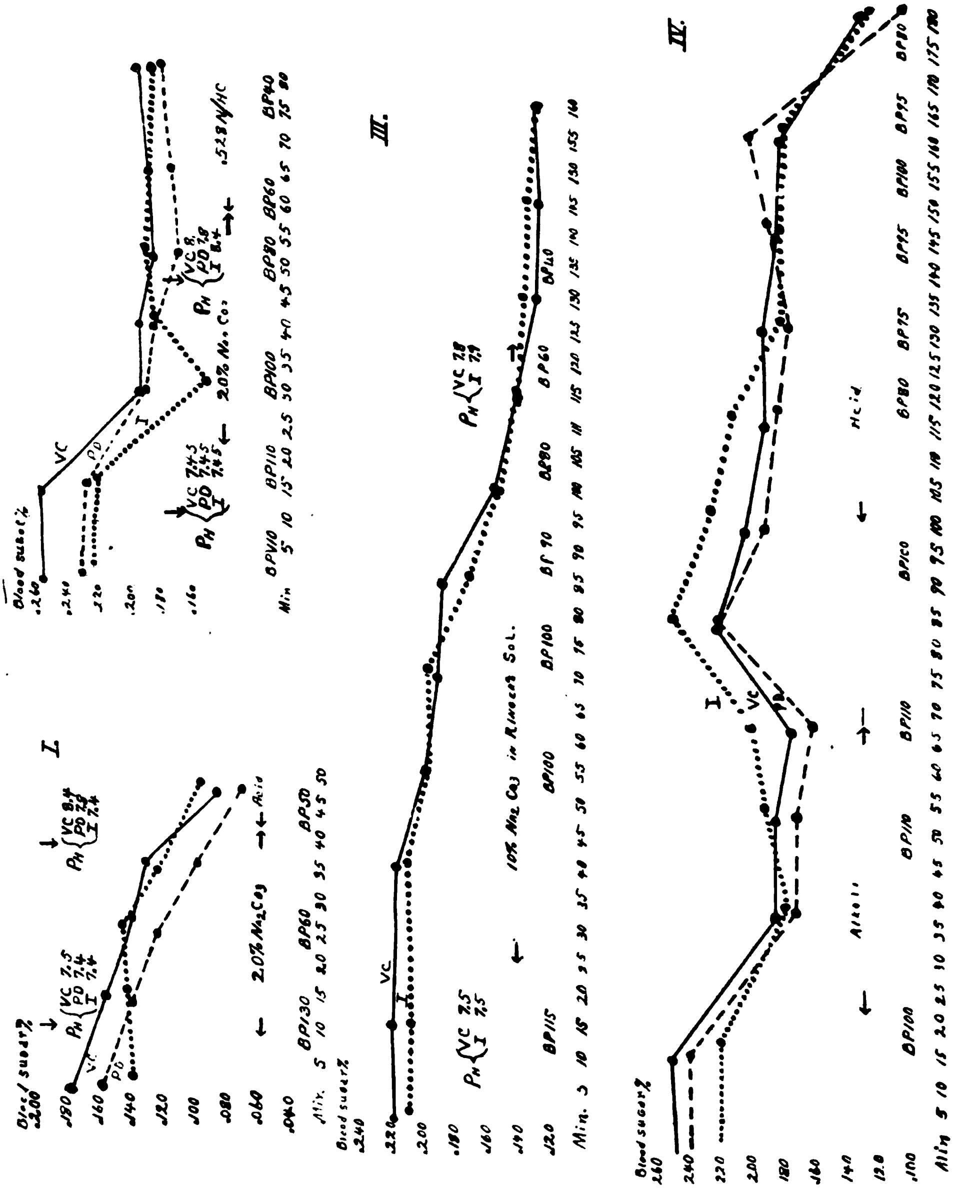
I
II
III
IV
Blood sugar %
Min.
VC
PD
I
P_H VC 7.5 PD 7.4 I 7.4
P_H VC 8.4 PD 7.8 I 7.4
20% Na₂CO₃
Acid
P_H VC 7.45 PD 7.45 I 7.45
20% Na₂CO₃
P_H VC 8. PD 7.8 I 8.4
.528 N/HC
P_H VC 7.5 I 7.5
10% Na₂CO₃ in Ringer's Sol.
P_H VC 7.8 I 7.9
Alkali
Acid
BP130
BP60
BP50
BP110
BP100
BP80
BP40
BP115
BP90
BP120
BP100
BP80
BP75
BP95
BP180
BP110

Fig. 1.

certain. Woodyatt's apparatus is, in our experience, unqualifiedly the best to use.

The sugar estimations were made by R. G. Pearce's modification of the Lewis-Benedict method (11), care being taken that the temperature conditions in the autoclave were the same for all the blood samples in the given experiment. This eliminates any error, in so far as comparative results are concerned, that might be incurred by an unequal degree of heating of different tubes.

Small samples of blood (about 5 cc. each) were also removed at intervals for the determination of the H-ion concentration by the dialysis colorimetric method of Levy, Rowntree and Marriott (12).

EXPERIMENTAL RESULTS

1. The hypoglycaemic influence of alkali. To study the hypoglycaemic influence of alkali, a strong solution of Na_2CO_3 was injected at constant rate into a small branch of the mesenteric vein of dogs to which, on the evening previous to the experiment, about 6 grams per kilogram of cane sugar had been administered by stomach tube. The results of these experiments are plotted in the four curves shown in figure 1.

Fig. 1. The influence of large injections of sodium carbonate on the sugar-content of blood from the pancreatico-duodenal vein, vena cava, and iliac vein. *V.C.*, Vena cava, continuous line; *P.D.*, pancreatico-duodenal vein, broken line; *I.*, iliac, dotted line; P_H, H-ion concentration; *B.P.*, arterial blood-pressure. The injections were made between the points of the arrows.

Experiment I. Sugar fed dog. Weight, 7.5 kg. Kennel fed plus 50 grams of cane sugar, given by stomach tube 20 hours before the collection of blood. Air pressure injection apparatus used, set to inject 50 cc. per hour. Solutions injected: (1) 20 per cent Na_2CO_3; (2) 0.346 N/HCl.

Experiment II. Sugar fed dog. Weight, 8.8 kg. Kennel fed plus 50 grams of cane sugar given by stomach tube 18 hours before collection of blood. Used Woodyatt's perfusion apparatus. Solutions injected: (1) 20 per cent solution of Na_2CO_3; (2) 0.528 N/HCl solution. Both solutions were injected at the rate of 1 cc. per minute.

Experiment III. Sugar fed dog. Weight, 7.8 kg. Kennel fed plus 46 grams of cane sugar, given by stomach tube, on each of two days previous to that of the experiment. Used Woodyatt's perfusion injecting apparatus. Solutions injected: (1) 10 per cent Na_2CO_3 and 0.528 N/HCl. Both solutions were injected at the rate of 1 cc. per minute.

Experiment IV. Sugar fed dog. Weight, 21 kg. Kennel fed plus 100 grams of cane sugar, given by stomach tube 20 hours before the collection of blood. Used Woodyatt's perfusion injection apparatus. Solutions injected: (1) 10 per cent Na_2CO_3; (2) 0.528 N/HCl. Both solutions were injected at the rate of 1 cc. per minute.

In experiment I the alkaline solution (20 per cent Na_2CO_3) was injected rapidly (considerably more than 1 cc. a minute). In II the same strength of alkali was more slowly injected (1 cc. a minute). In III and IV a 10 per cent solution of Na_2CO_3 was employed, the blood pressure being well maintained in IV, but falling fairly rapidly in III.

In Experiments I, II, III, the H-ion concentration of the blood, indicated by the figures standing opposite PH, was very considerably lowered by the injections, and this was no doubt also the case in IV, although PH was not determined in this experiment. In the first two experiments the alkali had a marked depressing influence on the animal (as indicated by the behavior of the arterial blood pressure), death occurring in one of them after about forty minutes. This depression was less marked in animals 3 and 4. The addition to the blood of such large quantities of alkali as were employed in these experiments is obviously far in excess of what could be tolerated therapeutically. Such quantities were used in order to ascertain to what extent a marked increase in the alkalinity of the blood and tissues could reduce the sugar-concentration of the blood.

A steady decrease in blood sugar is evident in all the experiments. This decrease might be due to a depression of the sugar-output by the liver, to increased sugar-retention or destruction by the muscles, to leakage of the sugar into the secretions, or to destruction of the sugar in the blood itself. Regarding the first possibility, there is some evidence of decrease in the sugar-output by the liver in Experiment II, but since no such evidence is forthcoming in the three other experiments of this group, increased retention of sugar by the liver cannot be considered as an important factor in explaining the decrease. Nor does increased retention of dextrose by the muscles appear to have occurred; indeed, on the contrary, in most of the experiments the blood sugar of the iliac vein after the injections had been continued for some time was somewhat higher than in that of the vena cava or portal vein. We are unable to offer any explanation of this unexpected result (loss of water?) The third possibility, namely, an escape of dextrose into the secretions, is supported by the lower percentage in the portal-blood than in either of the other bloods. This secretion may have occurred by way of the urine or intestine. The decreased glycosuria produced by alkali administration that has been observed by Murlin and Underhill would seem to support the idea that escape had occurred by the intestinal pathway.

2. Comparative sugar-retaining ability of liver and muscles during the injection of moderate amounts of dextrose into the portal vein. In two experiments (fig. 2) the sugar-content of blood removed from the portal vein, vena cava and iliac vein was determined at frequent intervals during the continuous injection of moderate amounts of sugar into the portal vein. In one (No. VIII) just enough sugar (viz., 0.14 grams per kilogram an hour) was injected in acid solution to cause a slight hyperglycaemia; whereas in the other (No. VII), the sugar was given in larger amounts (0.7 gram per kilogram an hour), and at intervals 10 cc. of a 15 per cent solution of sodium carbonate were injected through the pancreatico-duodenal vein. The results of the former experiment would seem to indicate that a slight degree of acidosis does not disturb the relationship of the sugar content of the blood from the three veins. In the latter experiment, the considerably larger, though not excessive, sugar injections caused the portal blood-sugar to rise above that of the vena cava, the muscle blood-sugar being distinctly lower. In this experiment it will also be observed that the H-ion concentration of the blood became raised in spite of the injection of considerable quantities of alkali. In both experiments the rise in blood-sugar was very distinct immediately following the injection (horizontal arrows), a practically constant level being attained after about thirty-five minutes when 0.7 gram dextrose per kilogram an hour was injected, and in about twenty minutes with 0.14 gram.

3. Comparative sugar-retaining ability of liver and muscles during the injection of large amounts of dextrose into the portal vein. The rapidity with which the excess of dextrose disappears from the blood when large amounts are suddenly injected intravenously has in general been found by previous workers to depend much more on retention of the dextrose by the muscles and other tissues than by the liver (Bang, Kleiner, etc.). Although there can be no doubt that the sudden flooding of the organism with dextrose in such experiments is to be considered as a highly abnormal condition, and one which cannot conceivably occur even during the severest forms of experimental hyperglycaemia, yet the results of the experiments have been thought to be of some value as indicating the manner by which *any* excess of dextrose in the blood might be dealt with. By comparison of the percentage of sugar in blood taken from the portal vein, the vena cava, and the iliac vein, we have studied the sugar-retaining powers of the liver and muscles during the continuous injection (by means of Woodyatt's apparatus) of from 2.4 to 3.5 grams dextrose per kilo body weight

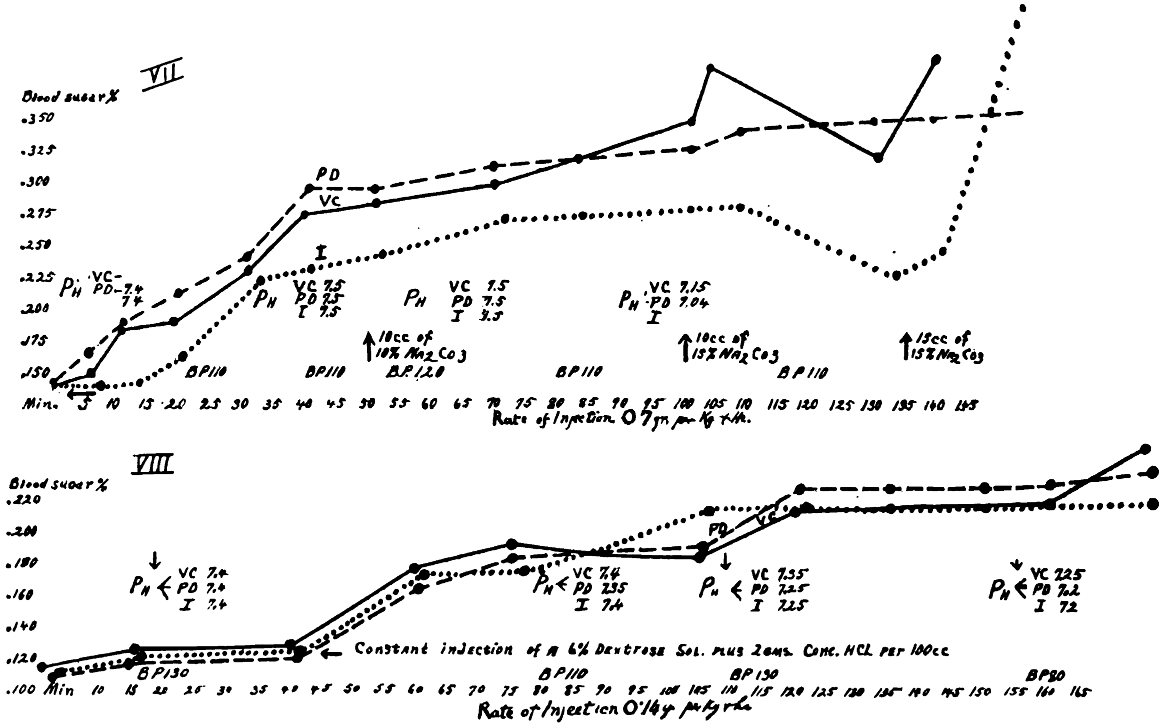

Fig. 2. Injections of moderate amounts of dextrose and of acid or alkali into the portal vein.

Experiment VII. Weight, 14.7 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Constant injection of a 6 per cent dextrose solution, 3 cc. per minute for 2 hours, 37 minutes, or 0.7 gram per kilo per hour. The vertical arrows indicate the time at which the alkaline solutions were injected.

Experiment VIII. Weight, 25.9 kg. Used Woodyatt's perfusion apparatus. Constant injection of an acid dextrose solution containing 2 grams of concentrated HCl per 100 cc. of a 6 per cent dextrose solution. Injected nearly 1 cc. per minute for 2 hours, 15 minutes, 0.14 gram per kilo per hour. Symbols same as in previous figures.

per hour. Acid or alkali was also injected at intervals through the pancreatico-duodenal vein. The results of the three experiments are depicted in the curves of figures 3, 4 and 5.

In Experiment XIII, figure 3, 3.5 grams dextrose per kilogram an hour was injected, causing a steady rise in blood-sugar in all three veins, most marked in the portal vein, next in the vena cava, and least in the iliac. As judged from the curves, the sugar-retaining power of liver and muscles is about equal. It will further be noted that, at intervals of about thirty minutes throughout the experiment, 10 cc. of 10 per cent sodium carbonate solution was injected through the pancreatico-duodenal vein, but that the H-ion concentration of the blood collected some minutes after several of these injections had been made did not show any increase in alkalinity; on the contrary, there was towards the end of the experiment a very distinct decrease in this regard, i.e., P_H became lower. Since in our other experiments approximately similar quantities of alkali have caused an increase in blood alkalinity, we have in the results of this experiment some evidence that, when large quantities of dextrose are injected into the blood, acid breakdown products result—presumably lactic acid. In work which we hope to publish immediately we have, as a matter of fact, evidence of a striking increase in the lactic acid content of the blood when much smaller quantities of dextrose in alkaline solution are injected.

In Experiment XIV, figure 4, injections of 3 grams dextrose per kilogram an hour, accompanied by injection by way of the pancreatico-duodenal vein of 10 cc. quantities of hydrochloric acid, gave results which differed from those of Experiment XIII in that the muscles showed no evidence of retaining any dextrose; on the contrary, during a great part of the experiment there was more dextrose in the blood coming from them than in that of the vena cava. Although estimation of the H-ion concentration of the blood could not be carried out in this experiment, there is no doubt, for the reasons given above, that it must have been raised. The effect of this on muscular tissue may have merely been to raise its power of absorbing water, thus causing an increase in the sugar concentration of the outflowing blood. We were well aware of this possibility when the experiment was performed, and attempted to control it by enumeration of erythrocytes, but unfortunately in the rush of work entailed in such experiments, failed to carry through the process satisfactorily.

In Experiment XV, figure 5, 2.4 grams dextrose per kilogram an hour was injected without any acid until after a little over an hour,

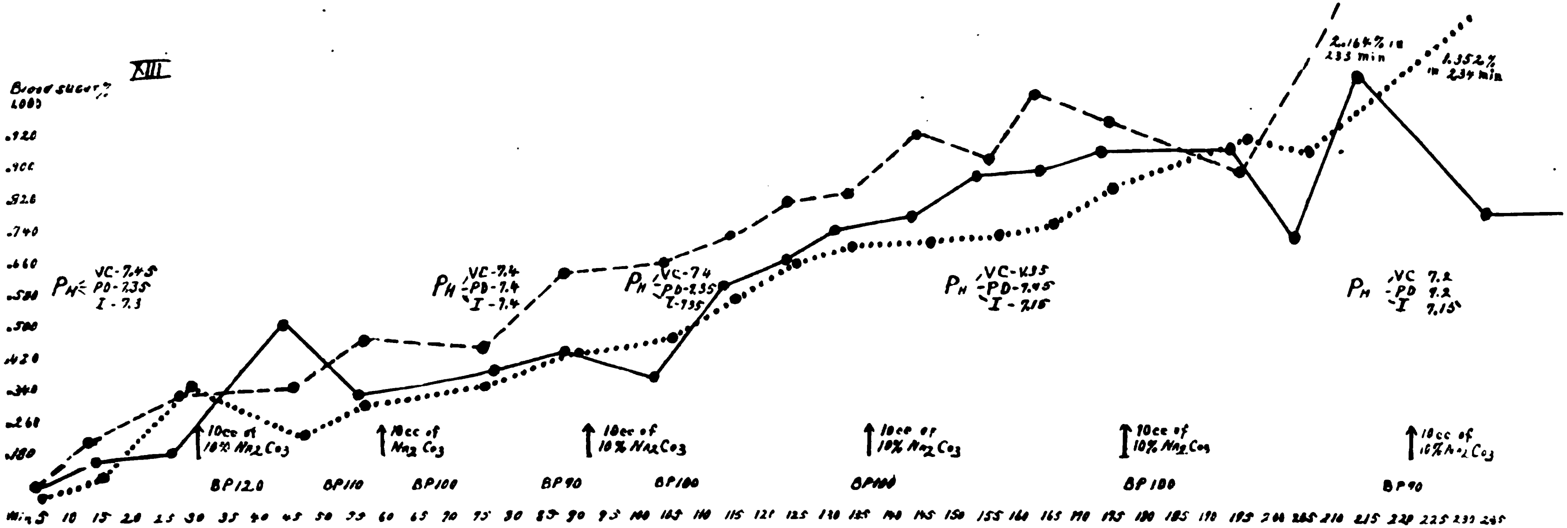

Fig. 3. *Experiment XIII.* Weight, 12.3 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Constant injection of an 18 per cent solution of dextrose, injecting 806½ cc. of the dextrose solution in 3 hours, 48 minutes, an average of 3.53 cc. per minute, or 3.5 grams per kilo per hour. At time indicated by vertical arrows Na_2CO_3 was injected into the pancreatico-duodenal vein. *V.C.*, Vena cava, continuous line; *P.D.*, pancreatico-duodenal vein, broken line; *I*, iliac, dotted line.

when hydrochloric acid in 10 cc. quantities was injected at about hourly intervals through the pancreatico-duodenal vein. It will be observed that the H-ion concentration of the blood did not rise except in that of the iliac vein. This is the only experiment in which large sugar-injections without *excess* of alkali did not cause a distinct decrease in PH. Regarding the behavior of the sugar, it will be observed that the liver and muscles apparently retained about equal amounts. Towards the end of this experiment a sudden fall occurred in the blood-sugar of all three vessels (not shown in the curve), although the sugar injections were being maintained. This fall occurred about the same time as a fairly rapid fall in arterial pressure and immediately following an injection of very strong acid. It would seem to indicate a sudden leakage of dextrose, perhaps because of the onset of marked oedema. In these three experiments it will be observed that a constant level of blood-sugar was never attained.

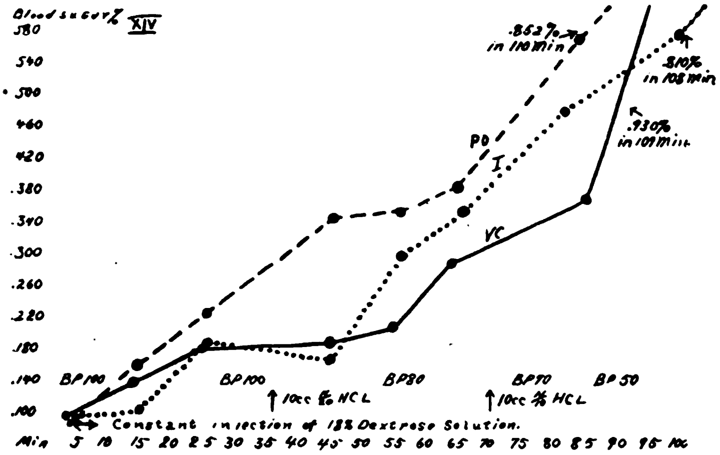

Fig. 4. *Experiment XIV*. Weight, 13.3 kg. Used Woodyatt's perfusion apparatus set at 2.5. Constant injection of an 18 per cent dextrose solution, injecting 3.8 cc. per minute for 1 hour, 30 minutes, or 3.08 grams per kilo per hour. At intervals specified above by the vertical arrows, 10 cc. of $\frac{N}{10}$ HCl and $\frac{N}{10}$ HCl was injected into the pancreatico-duodenal vein. *V.C.*, Vena cava, continuous line; *P.D.*, pancreatico-duodenal vein, broken line; *I*, iliac, dotted line.

4. The sugar-retaining ability of the liver during the injection into the portal vein of small quantities of dextrose in strongly alkaline as compared

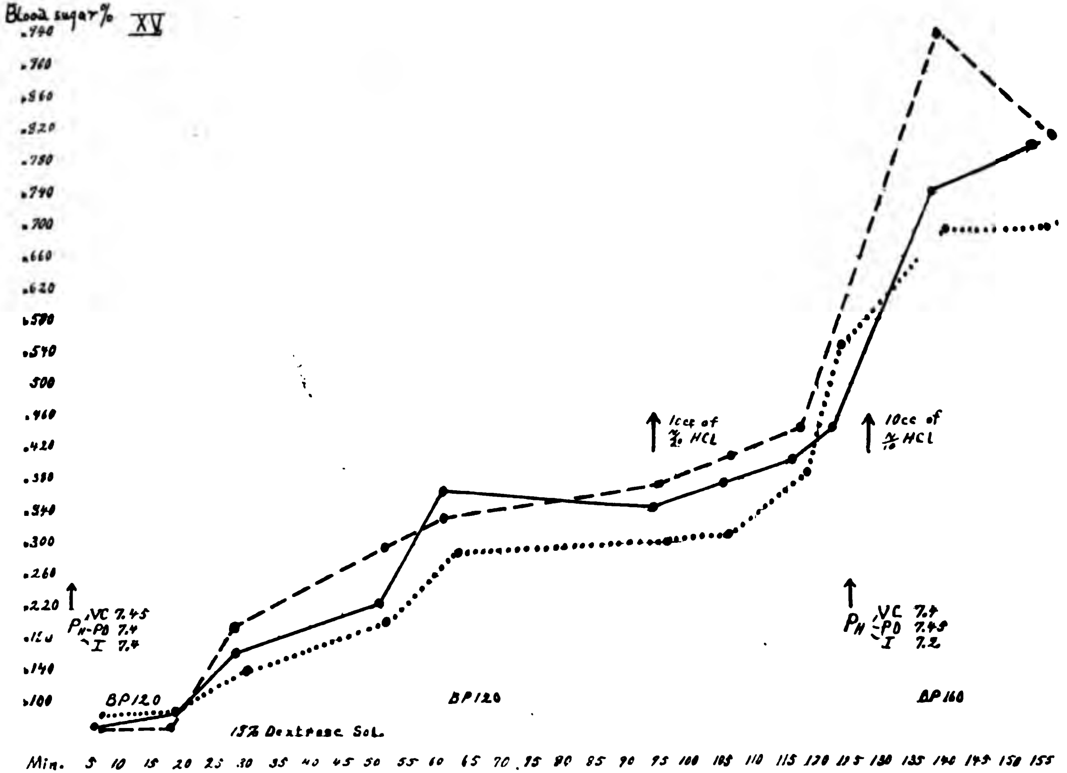

Fig. 5. *Experiment XV*. Weight, 17.2 kg. Kennel fed. Used Woodyatt's perfusion apparatus set at 2.5. Constant injection of an 18 per cent solution of dextrose. Injected 3.8 cc. per minute for 3 hours, 34 minutes, or 2.38 grams per kilo per hour. At intervals indicated by vertical arrows, acid was injected into the pancreatico-duodenal vein. *P.D.*, Pancreatico-duodenal vein, broken line; *V.C.*, vena cava, continuous line; *I.*, iliac, dotted line.

with neutral or faintly acid reaction. In the earlier experiments of this group (see fig. 6), the procedure consisted in collecting samples of blood from the vena cava and portal vein before and during the injection of a 4 per cent solution of dextrose into a mesenteric vein. The periods of injection were comparatively short, and the injections were made at a rate varying in the different experiments between 0.55 and 3.0 grams per kilogram an hour. The dextrose solution either contained from 10 to 20 per cent Na_2CO_3, or was neutral or faintly acid in reaction. Taking the results in general, it appeared as if the alkaline reaction caused a very striking sugar-retention as compared with that occurring with neutral or faintly acid dextrose solutions. Besides the table of results which we have already published elsewhere (14), the accompanying curves will show our reasons for coming to this preliminary conclusion. On closer examination of the curves, however, it will be observed that the above mentioned difference depends not so much on a falling away in the percentage of sugar in the cava blood as on the fact that the increase in the sugar-concentration of the portal blood produced by the injection of sugar solution is much more likely to occur suddenly when the latter is alkaline in reaction than when it is neutral or acid. When a similar rise in the portal blood does occur with neutral or acid sugar solutions, the liver retains just as much dextrose as it does in the presence of alkali. Another objection to the earlier experiments rests on the fact that the periods of injection were too brief, the one being also too quickly followed, sometimes without any no-injection interval, by the other.

As to the cause of the sudden increase in the portal blood sugar during alkali-dextrose injections, we are inclined to the belief that it was due to inequalities in injection rate, for although the same "constant-pressure" injection apparatus was employed as in previous work with 4 per cent dextrose solutions by one of us and R. G. Pearce, yet in the present experiments a lower pressure was employed, and this, along with the viscosity of the carbonate solutions, and perhaps on account of an action of the latter on the blood or blood vessels, produced a temporary block, which afterward suddenly gave way.

In any case we are certain that the marked increase observed in portal blood sugar in these experiments is due to some irregularity of injection rather than to any chemical action which the alkali might conceivably have in increasing the reducing power of the blood. Although at room temperature and for the brief period of time during which the alkali and dextrose solution stood prior to injection, we could

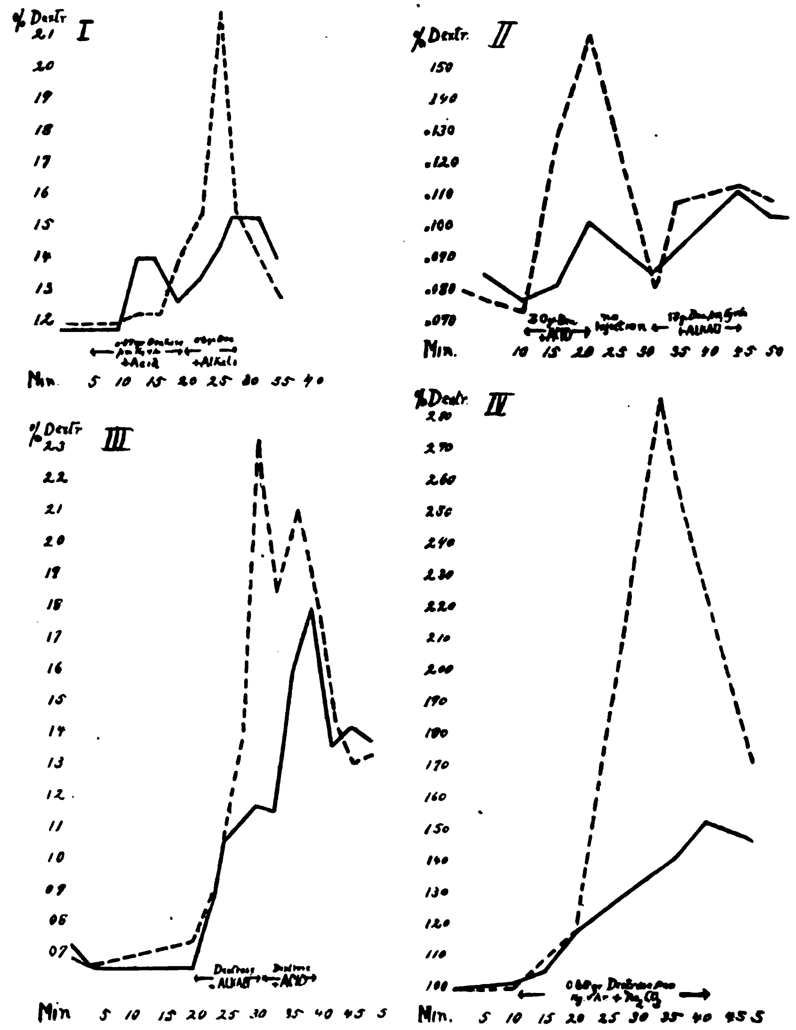

Fig. 6. The influence of alkali and acid on the blood-sugar in portal vein and vena cava during the injection of dextrose into the former. Broken line, blood of portal vein. Continuous line, blood of vena cava.

In I, 20 per cent Na_2CO_3 was injected at rate of 2.5 cc. per minute into portal.

In II, 5 per cent Na_2CO_3 was injected at rate of 7.5 cc. per minute and 0.5 per cent HCl at rate of 15.5 per minute into portal vein.

In III, 20 per cent Na_2CO_3 was injected, 8 cc. per minute. Weak (?) HCl at same rate.

In IV, 10 per cent Na_2CO_3 was injected, 2.6 per minute.

detect no change in its reducing power as measured by the Lewis-Benedict method, yet, since the changes described by Lobry de Brun, von Ekenstein and Nef must have set in, the possibility was considered as to whether the resulting mixture of hexoses, etc., might not, when mixed with blood, cause the production of strongly reducing substances. To test this possibility we added quantities of alkaline dextrose solutions similar to those used in the above injections to freshly drawn unclotted blood, and determined the reducing power after keeping the mixtures at body temperature for periods varying from a few seconds to over an hour. Since it is known that the products of the sugar-breakdown by alkali vary considerably according to whether or not oxygen is present, the above mentioned experiments were performed with both venous and arterial blood, the incubation in the former case being conducted in vacuo and in the latter, in the presence of air. It was found that, apart from slight differences in glycolysis, there was no difference in the sugar content of the various samples according to whether an alkaline or a neutral (or faintly acid) dextrose-solution was added to them.

We have repeated the injection experiments of this group, using the perfusion apparatus of Woodyatt, so that there could be no doubt as to constancy of injection, and with the modification that between the sugar-injection periods a sufficient interval was allowed to elapse during which the blood sugar could return to its normal level. The results are depicted in the curves of figure 7.

Taking as our criterion of the sugar-retaining power of the liver the difference between the portal and cava curves, it will be seen that with dextrose alone the percentage of blood-sugar in both veins is either the same (No. VI), or when the injection is somewhat greater, the portal vein sugar rises above that of the vena cava (No. VII). When acid sufficient to raise the H-ion concentration of the blood was given along with the dextrose, it was possible out of four experiments to demonstrate a greater percentage of sugar in the blood of the vena cava in only one case (No. IV). In the three other experiments of this type (Nos. II, III, V), the concentrations of sugar in the blood of these two veins were practically the same. Unfortunately in only one of these experiments (No. IV) was P_H determined at sufficiently frequent intervals to show that enough acid was being injected to cause a perceptible increase in the H-ion concentration of the blood. Since however the animals were all of approximately the same size, and the same amounts of acid were injected in all four experiments, it may be inferred that the blood in all cases showed a distinct degree of acidosis.

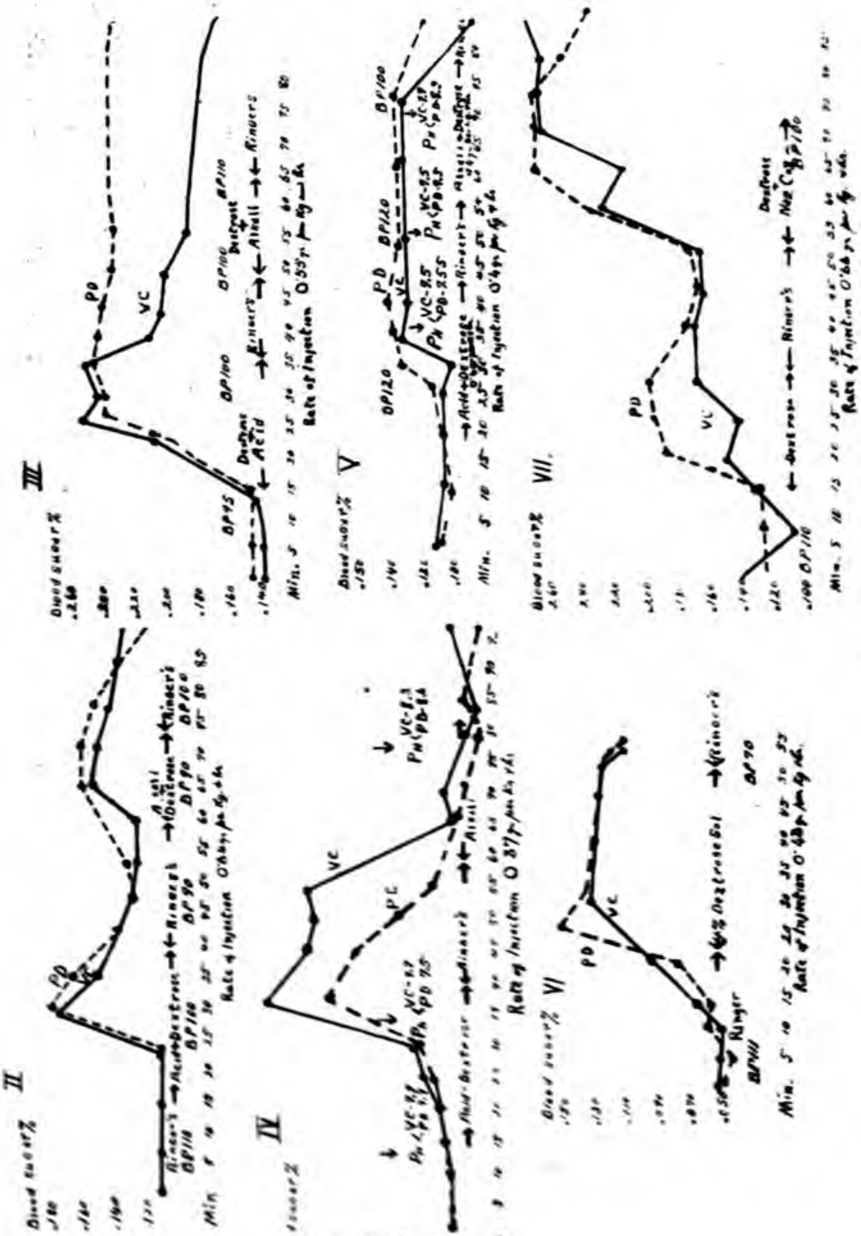
II
III
IV
V
VI
VII
Blood sugar %
PD
VC
Ringer's
Dextrose
Acid
Alkali
Min.
Rate of Injection

Fig. 7.

Experiments IV and V are strictly comparable in practically every detail and yet in the only one of them is there evidence that the acid injection stimulated the glycogenolytic process of the liver sufficiently to make the concentration of sugar in the cava blood greater than that in the portal in spite of sugar injections into the latter. When acid is injected without sugar, the results are similarly irregular (i.e., sometimes only does the cava blood-sugar overstep that of the portal). The conclusion which we draw from these results is that it is impossible to demonstrate with certainty in anaesthetised animals that increased acidity of the blood supplying the liver excites this organ to more active glycogenolysis.

Turning now to the influence of alkali which was investigated in five of the experiments, it will be noted in the first place that the sudden increases found to occur in the portal blood-sugar in the earlier experiments (fig. 7) were not observed, thus indicating, as already remarked, that these must have been due to some irregularity in the injection. In three of the experiments (viz., II, III, and IV) when injected along with alkali, the dextrose caused a distinctly lower degree of hyperglycaemia to become developed than when injected in acid or neutral reaction. In a fourth experiment (viz., No. 5), this depressing

Fig. 7. The influence of acid and alkali, as compared with Ringer's solution, on the blood-sugar in portal vein and vena cava during the injection of dextrose into the former. Lettering the same as in figure 1.

Experiment II. Weight 14.1 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 6 per cent dextrose solution containing 3 grams of concentrated HCl per 100 cc., and (3) 6 per cent dextrose solution containing 15 grams of Na_2CO_3 per 100 cc.

Experiment III. Weight, 10.5 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 4 per cent dextrose solution containing 2 grams concentrated HCl per 100 cc., and (3) 4 per cent dextrose solution containing 15 grams of Na_2CO_3 per 100 cc.

Experiment IV. Weight, 16.1 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 4 per cent dextrose solution plus 2 grams concentrated HCl per 100 cc. and (3) 6 per cent dextrose solution plus 15 grams of Na_2CO_3 per 100 cc.

Experiment V. Weight, 14.7 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 4 per cent dextrose solution plus 2 grams concentrated HCl per 100 cc., and (3) 4 per cent dextrose solution containing 10 grams Na_2CO_3 per 100 cc.

Experiment VI. Weight, 17.2 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of Ringer's and 4 per cent dextrose solution.

Experiment VII. Weight, 13.5 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's solution, (2) 6 per cent dextrose solution, and (3) 6 per cent dextrose solution containing 10 grams of Na_2CO_3 per 100 cc.

influence of alkali was somewhat later in showing itself, and in the fifth, (No. VII), in which however the amount of dextrose injected came very close to the 0.8 limit set by Woodyatt, etc., it was absent.

There can be no doubt from these results that alkali causes injected sugar to disappear more rapidly from the blood than otherwise would be the case, but there is only one experiment of this group (No. III) in which this disappearance might be attributed to a stimulation of the glycogen-retaining ability of the liver.

CONCLUSIONS

1. When dextrose is injected by way of the portal vein into anaesthetised dogs in amounts which are below the tolerance limit (about 0.8 grams per kilogram an hour), there occurs a rapid increase in the percentage of blood-sugar. This rise continues until a certain level is attained, after which the blood-sugar remains practically constant. The time at which the constant level is attained varies with the rate of injection; thus, with from 0.14 to 0.4 grams per kilogram an hour it was attained in twenty to twenty-five minutes (figs. 2 and 7), whereas with 0.7 gram it required about thirty-five minutes (fig. 2). When the injections are considerably above the tolerance limit, the increase in blood-sugar goes on steadily for a very long period of time (two or three hours). Unfortunately we can offer no data regarding the behavior of the curve for dextrose injections that are just above the tolerance limit, i.e., between 0.8 gram and 2 grams per kilogram an hour.

2. When the behavior of the sugar concentration is compared in blood drawn from the portal vein, vena cava and iliac veins during moderate dextrose injections, it has usually been found that there is very little difference. The sugar-retaining powers of the liver and muscles are therefore not sufficiently developed to cause any perceptible change in sugar-percentage in the inflowing and outflowing blood of these tissues when physiological quantities of dextrose are being absorbed. Although such retention is no doubt going on, it is too slow in relation to the large volume of blood circulating through the tissues to make any impression on the sugar-percentage. When large injections of dextrose are given, a distinct difference in sugar-concentration usually becomes evident, the higher percentage being in the blood of the portal vein, the next in that of the vena cava, and the lowest in that of the iliac vein, and the difference between the concentrations of blood-sugar in portal vein and vena cava is usually about the same as that in vena cava and iliac vein. This suggests

that the sugar-retaining power of the liver is approximately equal to that of the muscles of the hind limb.

3. During the injection of large amounts of dextrose the H-ion concentration of the blood, as determined by the colorimetric method, very commonly becomes increased. When alkali (Na_2CO_3) is injected intravenously in sufficient amount to lower the H-ion concentration of the blood, in animals in whom the blood-sugar percentage is initially high (etherization and operative manipulation in sugar-fed dogs), there is a very distinct decrease in blood-sugar, but by comparison of the curves which represent this in the portal vein, vena cava and iliac vein, it cannot be shown whether the liver or the muscles are primarily responsible for the increased retention. The three curves decline at an equal rate.

4. The injection of sodium carbonate solution or of hydrochloric acid at intervals into the portal vein during the continuous injection into another branch of the same vein of a dextrose solution does not disturb the relationship in sugar-concentration normally existing in blood taken from portal vein, vena cava and iliac vein, nor do such injections cause any alteration in the level of the curves, whether these be rising or stationary. When dextrose is injected at a rate below the tolerance limit, along with sufficient alkali to cause a distinct increase in P_H of the blood of the portal vein and vena cava, the blood-sugar does not rise to such a degree as it does when the dextrose solution is neutral or acid in reaction.

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THE RESPONSE OF THE VASOMOTOR MECHANISM TO DIFFERENT RATES OF STIMULI[1]

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Two effects are commonly observed upon stimulating the central end of a cut sensory nerve; (1) reflex pressor or vasoconstriction, (2) reflex depressor or vasodilation. Hitherto, considerable attention has been paid by investigators to the effect that different strengths of stimuli have upon the vasomotor center but few noted the effect that different rates of stimuli have upon this same mechanism.

Since it is possible to produce two effects, opposite in character upon stimulating the same sensory nerve, experimenters proceeded to find methods by which they could isolate the one or the other response. Latschenberger and Deahna (1) in 1816 were the first to obtain a fall in arterial pressure upon stimulating the central end of a cut sensory nerve other than the depressor from the aorta and heart. From their results, they attributed this fall in blood pressure to the use of weak stimuli and to certain changes in the vasomotor center itself. A fall in blood pressure was also recorded by Knoll (2) in 1885 upon stimulating sensory nerves. The results of his experiments led him to believe that a depressor response was usually the result of a weak stimulus whereas the pressor response followed, as a rule, a stronger stimulus. Upon stimulating muscles mechanically, by massage, Kleen (3) remarked a fall in general blood pressure.

Howell, Budgett and Leonard (4) were able to obtain reflex vasodilator and reflex vasoconstrictor responses from the same nerve trunk by cooling the nerve to 0°C. central to the point of stimulation. A fall in pressure was always the result of this procedure. This method, along with others, was also employed by Hunt (5). He obtained, upon stimulation, depressor responses from all sensory nerves tested, except the saphenous, in the cat; (a) when the nerves were cooled to 1°C.; (b) when the nerves were cut and recently regenerated; (c) when mechanical stimuli were applied to muscles; (d) when the electrical stimuli were weak; and (e) when the nerves were subjected to repeated stimulation over a long period. His experiments were recently repeated by Vincent and Cameron (6) who obtained similar results. They attribute a part of the fall in blood pressure to the respiratory movements. A simple inductorium was used in these experiments and the strength of the stimulus was increased or decreased by increasing or decreasing the number of cells in the primary circuit.

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[1] Reported at the 1915 meeting of the American Physiological Society. This Journal, 1916, xl, 136.

A fall in blood pressure was noted by Martin and Lacey (7) in all the experiments performed, except one. The success in obtaining depressor responses, they attributed to the use of weak or threshold currents.

Stimuli applied peripherally to severed nerves arouse the one or the other vasomotor response according to the rate of interruption of the stimulating current. Ostraumoff (8) observed that when the rate of stimulation was slow there resulted a rise in the temperature of the limb (vasodilation) but when rapid there was observed a fall in temperature (vasoconstriction). Similar results were observed by Kendall and Lucksinger (9). Bowditch and Warren (10) plethysmographed the limb and observed that a slow rate 1 to 4 stimuli per second was favorable to vasodilation and 30 to 64 stimuli per second favorable in producing vasoconstriction. Bradford (11) remarked similar results upon the vasomotor nerves to the kidney.

Since different rates of peripheral stimuli are favorable for isolating the different vasomotor responses, is it not possible that such changes could be effected reflexly? Few investigators applied different rates of stimuli centrally to sensory nerves when testing vasomotor reflexes. Hunt (5) is, as far as I know, the only investigator employing different rates who states the resultant responses. He employed rates varying from 1 per two seconds to 60 per second. In his research, the rate of stimulation did not alter the character of the vasomotor response. Martin and Lacey (7) tried rates of stimuli varying from 2 to 8 per second with one form of apparatus and from 4 to 60 per second with another. They, however, do not state which of these rates was commonly employed, nor do they state the effects observed upon changing the rate.

This research was undertaken to determine (a) whether or not the rate of stimulation has an effect upon the reflex vasomotor mechanism;

(b) whether or not the marked uniformity in the depressor effects observed by Martin and Lacey (7) may be due not only to the fact that they employed weak or threshold currents but also that they employed slower rates than did previous investigators.

THE METHOD

Cats, narcotized with urethane (2 grams per kilo, by stomach), were used in these experiments. A few experiments were also performed upon cats under ether anaesthesia. The skin was incised on the median line of the neck, and the animal tracheotomized. The blood pressure was registered usually from the right carotid but in a few cases from the femoral artery by means of a mercury manometer. A signal magnet, which marked intervals of five seconds, was placed at the atmospheric pressure line of the manometer. It also indicated in most cases the time of stimulation.

For sensory stimulation the saphenous, peroneal, ulnar, radial, median and popliteal nerves were used. By making a small slit through the skin, at the position of the nerve, the nerve was isolated, cut, and the central end fastened in a Sherrington (12) shielded electrode. The electrode was then held in place by fastening around it, with paper clips, the two flaps of skin. The nerves were always used for stimulation in the order named above.

THE STIMULATING CURRENT

1. *The strength.* The strength of the stimulating current was usually 0.1 ampere in the primary circuit, and the strength of the secondary current was determined in Z units according to the Martin (13) method. In a few cases 1.0 ampere in the primary circuit was used. Since the electrodes were not altered after once in place and comparative results were desired this method was thought to be sufficiently accurate. Tissue resistance and β units were, therefore, ignored.

2. *The rate.* A glass knife blade key (13) moved back and forth by a motor, made and broke the primary circuit uniformly one to twenty times per second. A slower rate 1 per two seconds was obtained by interrupting the current by hand with the same key. No attempt was made to short circuit the make shock. This alternating effect was thought to overcome any polarization which might take place in the nerve trunk at the point of stimulation.

RESULTS

For convenience, the results are collected under three general heads according to the strength of the stimulus used. In table 1 the strength of stimulus is weak, it is about the same as that employed by Martin and Lacey (7): 5.8 to 17 Z units. Moderate strengths were employed in table 2. These varied from 32 to 224 Z units. In table 3 one strength of current, 494 Z units, was used.

TABLE 1

Blood pressure changes induced by altering the rate of stimulation on sensory nerves in 16 cats under urethane anaesthesia. Strength of stimulus 5.8 to 17 Z units

NERVE	NUMBER OF READINGS	AVERAGE Z UNITS	RATE OF INTERRUPTION OF CURRENT PER SECOND								
			4	8			20				
			−	−	−+	+−	−	−+	+−	+	0
Saphenous	15	8.4	15	11	3	1	3	5		7	
Peroneal	18	12.2	18	17	1		3	8	1	5	1
Ulnar	7	13.1	7	7			4	2		1	
Radial	2	9.7	2		2					2	
Median	3	13.5	3	3			1	2			
	45	11.2	45	38	6	1	11	17	1	15	1
Per cent			100	84	13	3	25	38	2	33	2

In this and following tables − fall in blood pressure; + rise in blood pressure; −+ fall followed by a rise; +− rise followed by a fall; 0 no change.

In table 1, 45 serial readings were made upon 16 animals. In this series of experiments at 4 interruptions per second 100 per cent of the experiments gave a pure fall in arterial pressure. This per cent was decreased to 25 upon changing the rate of stimulation from 4 to 20 interruptions per second. Usually the fall produced by 20 interruptions per second was less than that at 4 per second. With 20 interruptions per second 33 per cent of the experiments presented a pure rise in blood pressure and 40 per cent of the experiments showed a fall either preceded or followed by a rise in arterial pressure (see table 1).

The data, 51 serial readings, in table 2 were collected from 21 animals. There is in this table a greater range in strengths of stimuli, than in table 1, 32 to 224 Z units. However, the reactions are similar. With a rate of interruption of 4 per second 90 per cent of the readings gave a pure fall, 6 per cent a fall followed by a rise and 4 per cent a pure rise in arterial pressure. With 20 interruptions per second 21 per cent of the readings presented a fall, 24 per cent a fall followed by a rise and 55 per cent of the readings gave a pure rise in blood pressure (see table 2).

Slower rates were employed in table 3. In most instances 1 per second was employed for the slowest rate in the series but occasionally 1 per two seconds was used. With this rate of stimulation 93 per cent of the readings presented a fall, 5 per cent a fall followed by a rise and 2 per cent a rise in arterial pressure, with the rapid rate of interruptions—20 per second—only 2 per cent gave a fall in blood pressure; 11 per cent of the readings presented a fall followed by a rise, 11 per cent a rise followed by a fall and 76 per cent a pure rise in blood pressure (see table 3).

TABLE 2

Blood pressure changes induced by altering the rate of stimulation on sensory nerves in 21 cats under urethane anaesthesia. Strength of stimulus 32 to 224 Z units

NERVE	NUMBER OF READINGS	AVERAGE Z UNITS	RATE OF INTERRUPTION OF CURRENT PER SECOND									
			4			8				20		
			−	−+	+	−	−+	+	0	−	−+	+
Saphenous	13	49	13			8	4	1		2	3	8
Peroneal	25	67	21	3	1	12	9	4		7	3	15
Ulnar	9	135	8		1	7		1	1	1	4	4
Median	4	79	4			3	1			1	2	1
	51	82	46	3	2	30	14	6	1	11	12	28
Per cent			90	6	4	60	27	11	2	21	24	55

Some curves illustrative of the results obtained upon varying the rate of the stimulus are here presented. Figure 1 is a curve in which weak or threshold stimuli were used. In this record the strength of stimulus was 5.8 Z units. At (*1*) the saphenous nerve was stimulated at the rate of 20 per second. There resulted during the time of the stimulus (approximately 30 seconds) a rise of arterial pressure from 96 to 99 mm. of mercury—a rise of 3.1 per cent. At (*2*) the rate of stimulation was 8 per second. At (*3*) the rate of interruption was 4 per second and there resulted a fall in arterial pressure from 98 to 94 mm. of mercury—a drop of 4.1 per cent.

TABLE 3

Blood pressure changes induced by altering the rate of stimulation on sensory nerves in 24 cats under urethane anaesthesia. Strength of stimulus 494 Z units

NERVE	NUMBER OF READINGS	RATE OF INTERRUPTION OF CURRENT PER SECOND															
		1*			4				8				20				
		−	−+	+	−	−+	+−	+	−	−+	+−	+	−	−+	+−	+
Saphenous	14	13	1		5	7	1	1	1	6	1	6	1		1	12
Peroneal	22	21	1		8	5	4	5	3	1	2	16		3	3	16
Ulnar	12	10	1	1	4	5		3	3	1	1	7		2	1	9
Median	5	5			1	1	1	2	1	1	1	2		1	1	3
Popliteal	2	2			1	1				1		1				2
Radial	1	1				1						1				1
	56	52	3	1	19	20	6	11	8	10	5	33	1	6	6	43
Per cent		93	5	2	33	36	11	20	14	18	9	59	2	11	11	76

* In some cases 1 per two seconds.

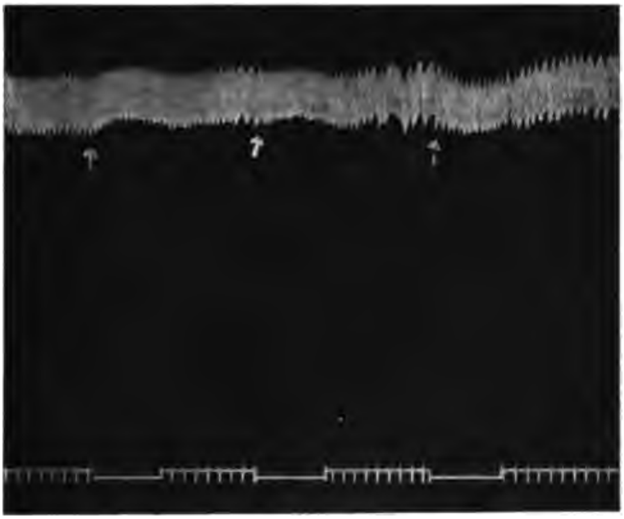

Fig. 1. Upper curve blood pressure. The lower line time in five seconds and the point of stimulation (also zero blood pressure). Saphenous nerve 5.8 Z units. This and all following records are explained in the text.

Figure 2 is a type of curve in which pure pressor and depressor results are observed. In this record the strength of stimulus applied to the saphenous nerve was 32 Z units. Similar results were obtained upon this animal with as weak a current as 17 Z units. From this curve the striking difference in the effects that slow and rapid rates have upon the vasomotor mechanism can be seen. At (*1*) the rate of stimulation was 4 per second and applied approximately 30 seconds. The arterial pressure decreased from 117 to 106 mm. of mercury—a fall of 9.4 per cent. At (*2*) the rate of stimulation was 20 stimuli per second and the blood pressure rose from 115 to 127 mm. of mercury—a rise of 10.4 per cent. Similar results were obtained upon other animals not only with moderately weak currents but also with threshold currents and with stimuli up to 283 Z units.

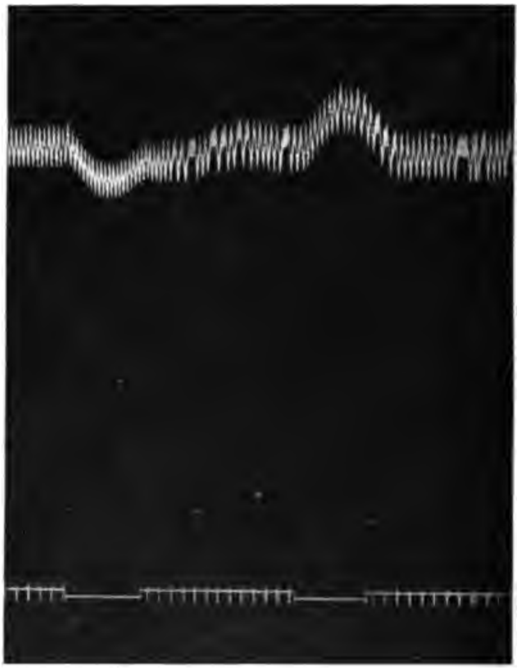

Fig. 2. Upper curve blood pressure. Lower line zero pressure, time in five seconds. Stimulation of saphenous nerve.

A reversal of the blood pressure is not always obtained upon increasing the rate of interruption but a fall produced by a rapid rate is usually less than that brought about by a slower rate of stimulation. Figure 3 is a curve obtained by stimulating the ulnar nerve with a strength of stimulus of 88 Z units. At (*1*) the rate of stimulation was 4 per second and there resulted a fall of blood pressure from 116 to 102 mm. of mercury—a drop of 12 per cent. Twenty interruptions per second were used in (*2*) and there resulted a fall of 2 mm. of mercury—a drop of about 2 per cent. Seven interruptions per second were used in (*3*) and 4 interruptions in (*4*). In the former case there resulted a fall of 4 per cent and in the latter a fall of 8.5 per cent.

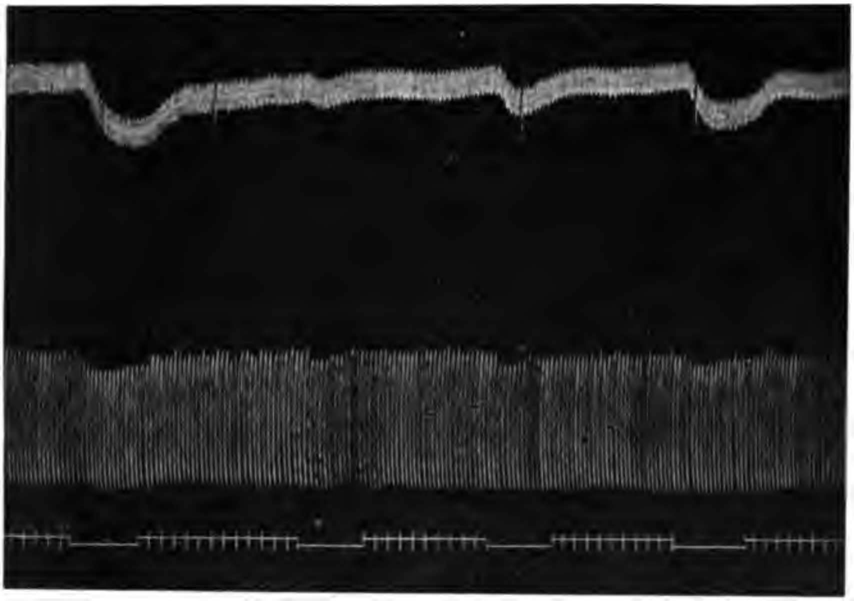

Fig. 3. Upper curve blood pressure. Middle curve respiration. Lower line zero blood pressure, time in five seconds. Stimulation of ulnar nerve.

Figure 4 is another type record obtained upon stimulating the peroneal nerve with a strength of stimulus of 32 Z units. At (*1*) the rate of stimulation was 7 per second and a fall in blood pressure is observed. In this curve as in other curves in which the rate of the stimulus was favorable to dilation, the pressure falls and rises slowly. When the rate is faster the curve falls abruptly and ascends rapidly and may reach the normal level before the stimulus has ceased. At (*2*) the rate of stimulation was 20 per second and there is seen a slight fall in blood pressure followed by a rise. This type of curve was frequently observed when the stimulus was weak or moderately strong. As the currents became stronger the curves were usually the reverse, that is, a rise was usually followed by a fall in blood pressure.

Figure 5 is a record obtained by stimulating the ulnar nerve with a strength of stimulus of 494 Z units. At (*1*) the rate of stimulation was 1 per second and there resulted a fall in arterial pressure of 13 per cent. At (*4*) the rate of stimulation was 4 per second and at (*20*) 20 per second, with a resultant rise in arterial pressure of 26 per cent in the latter case.

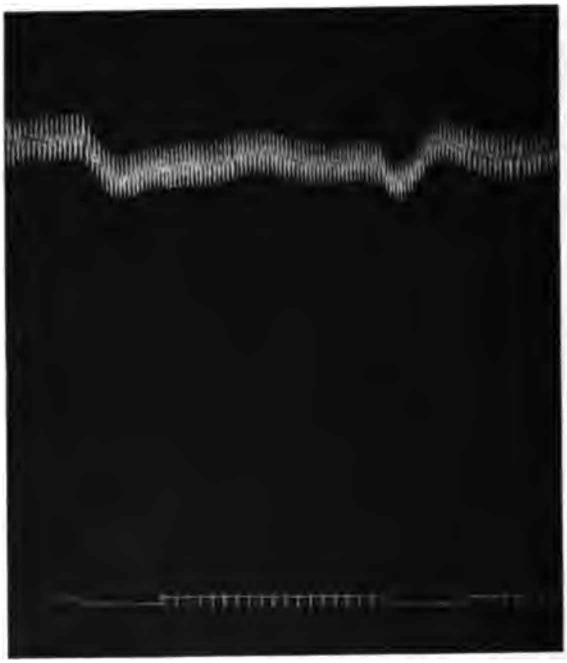

Fig. 4. Upper curve blood pressure. Lower line, atmospheric pressure, time five seconds, and point of stimulation.

Hunt (5) summarizes his results on changes in the rate of stimulation upon the vasomotor center as follows, "Only a rise in pressure resulted, the character of which was determined by the number and strength of the stimuli; the rise was very gradual with slow weak stimulation, but took place much more suddenly with stronger and more rapid stimulation."

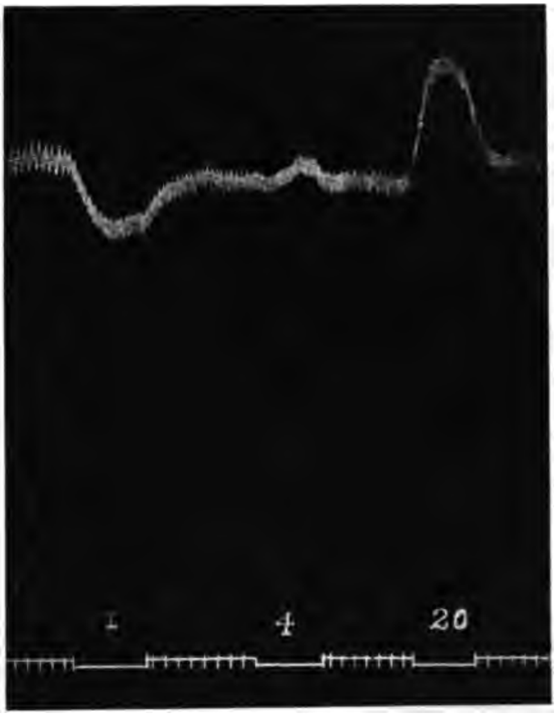

Fig. 5. Upper curve blood pressure. Lower line zero blood pressure, time five seconds, and point of stimulation.

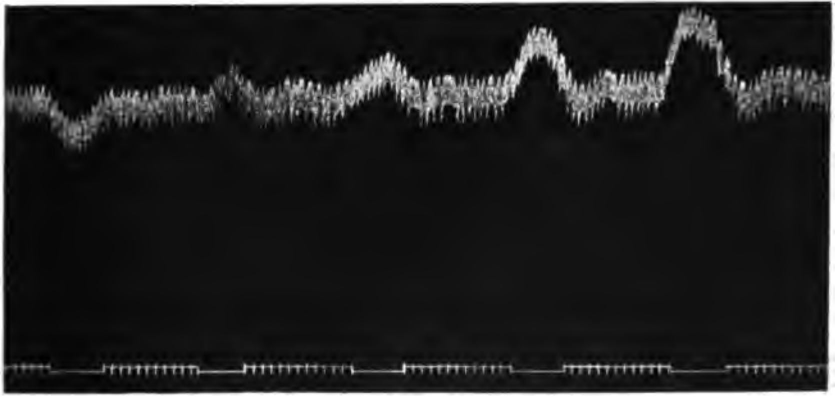

Fig. 6. Upper curve blood pressure. Lower line zero blood pressure, time in five seconds and point of stimulation.

The results obtained in figure 6 are slightly different from those observed by Hunt. The strength of stimulus applied to the peroneal nerve was 494 Z units. At (*1*) the rate of stimulation was 1 per second causing a fall of 11.3 per cent. At (*2*) the rate was 4 per second from which there resulted a rise of 7.1 per cent. At (*3*) the rate of stimulation was 8 per second producing a rise in arterial pressure of 11.2 per cent. A rise of 20 per cent was observed when the rate of stimulation was 16 per second (at *4*) and a rise of 28 per cent was obtained when the rate of stimulation was 20 per second (at *5*).

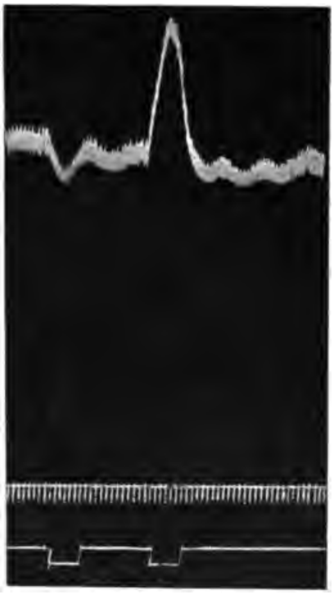

Fig. 7. Upper curve blood pressure. Middle line zero pressure and time in five-second intervals. The lower line the point of stimulation.

In a number of experiments a current of 650 Z units slowly interrupted (1 per second) gave a fall in pressure, whereas 4 per second produced a rise. In only two instances stronger currents were employed. In these cases 1 ampere was used in the primary circuit. One animal, on November 23, from which figure 7 is taken 2244 Z units produced a fall of 6 per cent when the rate of interruption was 1 per two seconds (at *1*) and a rise of 33 per cent when the rate of interruption was 4 per second (at *2*). A rise of 7 per cent in blood pressure was obtained on this same popliteal nerve, when the rate of interruption was 10 per second and the strength of stimulus was 244 Z units. Using the peroneal nerve of the same animal 8891 Z units interrupted at the rate of 1 per two seconds produced a fall of 5 per cent, and a rate of interruption of the stimulating current—20 times per second—produced a rise of 28 per cent. On November 26, similar results were obtained; 17 Z units at 20 interruptions per second produced a rise in blood pressure. The current was then increased in strength to 6643 Z units with a rate of 1 per two seconds a fall of 5 per cent resulted, when the current was 4 per second, a rise of 7 per cent was observed.

DISCUSSION

From the above data it is shown conclusively that not only the strength but also the rate of stimulation is important in bringing about reflex vasodilator and reflex vasoconstrictor changes. With the same strength of stimulus pressor and depressor results are obtainable by varying the rate of stimulation from 1 to 20 stimuli per second. A rapid rate of stimulation which usually brings about vasoconstriction occasionally produces a fall in blood pressure, but this fall is almost always less than that produced by a slower rate of interruption. With a slow rate, 4 per second, depressor (reflex vasodilator) responses, and with a rapid rate, 20 per second, pressor responses have been observed with 5.8 Z units. Upon increasing this current to 383 Z units or seventy times 5.8 Z units these same phenomena have been observed. Upon increasing the current to 494 Z units usually the rate of 4 per second produced a rise in pressure but a slower rate 1 per second or 1 per two seconds produced a fall. Not only was it possible to bring about a fall in blood pressure with 1 per two second stimulation with 494 Z units, but the strength of stimulus could be increased to 6643 Z units and still a fall was observed. This current was 390 times stronger than the current necessary to produce a rise in blood pressure when interrupted 20 times per second. That summation does take place with rapid rates of stimulation is undisputable, but it does not seem probable in this case where the strength is more than 400 times threshold that the phenomenon of summation can explain the different effects obtained with these rates of 1 per two second and 20 per second interruptions.

Most observers believe the pressor response to be the "normal" and predominating response upon cutaneous sensory nerve stimulation and the depressor response unusual except upon alteration in the nerve tissue itself (14). Martin and Lacey claim that the depressor response is the natural response because the threshold is lower. My experiments throw no further light upon the subject than that the threshold may be the same for both pressor and depressor responses, depending upon the rate of stimulation. If, however, the rate of stimulation is slow and uniform, then the threshold for the depressor response is the lower as observed by Martin and Lacey (7). Their claim (7) that depressor responses are not unusual, upon sensory nerve stimulation, is supported by these results.

SUMMARY

1. The rate of stimulation modifies the responses obtained reflexly from the vasomotor mechanism, when the saphenous, peroneal, ulnar, radial, median and popliteal nerves are stimulated centrally.

2. These results confirm Martin and Lacey's conclusions that no difference in effect on blood pressure was observed when different sensory nerves were stimulated. Radial, ulnar, median, peroneal, saphenous and popliteal nerves gave depressor responses upon stimulating with a weak, slowly interrupted current (4 per second). Pressor responses were obtained upon these same nerves with weak currents but the rate of interruption was increased from 4 to 20 per second.

3. Slow rates (1 per second or 1 per two seconds) of stimuli act favorably in bringing about depressor responses even when the strength of stimulus is 494 to 8891 Z units or 90 to 400 times the threshold stimulus.

4. In 45 serial readings made upon 16 cats under urethane anaesthesia, the average strength of current was found to be 10.2 Z units. One hundred per cent of the readings showed depressor effects with a rate of 4 stimuli per second and 73 per cent showed a pressor effect with a rate of 20 stimuli per second.

With an average current of 82 Z units for 51 readings upon 21 cats, 96 per cent of the readings showed a fall in blood pressure with a rate of 4 stimuli per second and 79 per cent showed a rise in arterial pressure with a rate of 20 stimuli per second.

With a strong current 494 Z units for 56 readings upon 24 cats 98 per cent showed a fall in arterial pressure at 1 per second or 1 per two second stimulation. Ninety-eight per cent of the readings showed a rise in blood pressure when the rate of stimulation was 20 stimuli per second.

5. Whether pressor or depressor responses are the "normal" results upon sensory stimulation depends upon the rate of stimulation and the strength of the stimulus. The threshold for both may be the same if the rate is slow (4 per second) for depressor and rapid (20 per second) for pressor responses.

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(11) **Bradford**: Journ. of Physiol. 1889, x, 390.
(12) **Sherrington**: Journ. of Physiol. 1909, xxxviii, 382.
(13) **Martin**: Measurement of induction shocks, New York, 1912, 60–69.
(14) **Hofmann**: Nagel's Handb. d. Physiol. des Menschen, Braunschweig, 1910, i, 322.

THE EFFECT OF TEMPERATURE CHANGES ON RHYTHM IN THE HUMAN ELECTROMYOGRAM

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INTRODUCTION

The frequency of nerve impulses discharged from the central nervous system in voluntary and reflex contractions of the skeletal muscles presents a problem concerning which great difference of opinion is found among investigators. Piper[1] who has made the most extensive study of action currents in human muscles, has shown with a string galvanometer that when electrodes are applied to the skin over a skeletal muscle (e.g., a forearm flexor) in such a way that interference currents are minimized, a fairly regular series of action currents with a rhythm of about 50 per second accompanies voluntary contraction. He states that the electrical effect of voluntary contraction can be reproduced by stimulating the motor nerve with 50 induction shocks per second, and that the records made under the two conditions are quite similar.[2] He infers from this that the central nervous system sends to the muscle 50 impulses per second.

Buchanan[3] has repeated the observations of Piper, using a capillary electrometer instead of a string galvanometer. She dissents, however, from Piper's conclusion that the muscle reveals the rhythm of innervation. Her reasons are in the main two. In the first place, her attempt to duplicate the electromyogram of voluntary contractions by stimulating the median nerve of a human subject with 50 induction shocks per second, yields with her capillary electrometer a record differing from that of the voluntary contraction.[4]

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[1] Piper: Elektrophysiologie menschlicher Muskeln, Berlin, 1912; Pflüger's Arch., 1909, cxxix, 145, etc.

[2] Piper: Arch. f. Physiol., 1910, 208.

[3] Buchanan: Quart. Journ. Exper. Physiol., 1908, i, 225.

[4] Loc. cit., 229 and 242, figs. 6 and 10.

Her second reason is based on an important series of experiments[5] with decerebrate frogs drugged with strychnine. She found that when the frog's gastrocnemius muscle was connected with the electrometer the records of the reflex spasm showed usually two distinct rhythms. There were large undulations with frequencies of from 3 to 14 per second, and superimposed upon the initial or ascending phase of each of these were smaller undulations with frequencies between 40 and 100 per second. The larger undulations she designates waves; the smaller, wavelets. By placing the gastrocnemius muscle in a moist chamber separate from the rest of the animal, except for an aperture to admit the nerve, she was able to raise and lower the temperature of the muscle without altering that of the nerve centers. The result of this procedure was to raise and lower correspondingly the frequency of the "wavelets" without changing that of the waves. The wavelet frequency at a muscle temperature of 7°C. varied from 40 to 54 per second, at 16°C. it lay between 100 and 110 per second. From this she argued that the wavelets do not correspond with any central nervous rhythm but indicate a rhythm intrinsic in the muscle. To show that the wavelet rhythm is completely independent of the nerve centers she altered the temperature of the spinal cord while keeping the temperature of the muscle constant, with the result that the frequency of the waves changed but not that of the wavelets. She believes that the undulations seen in the electromyogram of human muscles in voluntary contraction partake of the nature of the wavelets in her frog records rather than of that of the waves. Certainly their frequency would so suggest. She contends that as the "wavelet" rhythm is "of peripheral origin," we have no evidence that the central stimulus is discontinuous in its nature. She attempted in one case to extend her observations on change of temperature to human muscles by comparing the electromyogram of the same individual with the arm in an atmosphere at 13°C. in one case, and in an incubator at 35°C. in the other. She found no change of rhythm sufficiently marked to exceed the limits of variation resulting from the usual irregularity in the oscillations. It may be remarked here that the temperature-regulating powers of the circulation are probably sufficient to maintain a close approximation to normal temperature in the muscle fibers under such diversity of atmospheric temperature as was here employed. At least the change of temperature was probably insignificant compared to that imposed on the frog's muscles.

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[5] Buchanan: Loc. cit., 213–221; cf. also Journ. Physiol., 1901, xxvii, 95.

Garten[6] in dealing with this problem recorded human electromyograms under stimulation of the motor nerve with a constant current, with an alternating current of 2200 cycles per second, and in voluntary contraction. He found approximately the same action current rhythm under all three conditions, which suggested that the rhythm was intrinsically muscular and independent of innervation rhythm. But a comparison of the records obtained from the nerve and muscle of the rabbit, the nerve being in each case stimulated with a constant current, showed so close a correspondence of rhythm in the two tissues that he concludes[7] that the muscle probably affords a true record of the discharge of nerve impulses from the ganglion cells.

Piper[8] argues against Buchanan's view, contending that the characteristic rhythm of 50 per second in voluntary contraction must be determined by an equal frequency of nerve impulses because it is possible to raise the frequency of induction shocks applied to a motor nerve as high as 300 per second and still obtain a corresponding action current rhythm in the muscle. He argues that if the muscle is capable of responding separately to 300 nerve impulses a second it would not limit itself to 50 responses per second when subject to stimulation which is continuous or of a frequency too high for the muscle to follow. The argument appears at first sight convincing, but we believe we can show presently that it is not necessarily valid. Piper[9] has also reported an experiment on the progressive increase in frequency of rhythm accompanying progressive rise in temperature, but the experiment is not crucial since he raised the temperature of the entire animal (turtle), thus making the change common to both nerve center and muscle.

Dittler[10] has made an important contribution to the subject by recording with the string galvanometer the action current of the diaphragm of the rabbit in spontaneous respiration, and subsequently[11] the action current of the severed phrenic nerve under experimental conditions as nearly similar as possible. In these experiments the rhythm of the muscle corresponded with that of the nerve so closely that Dittler concluded that in general a muscle reproduces the rhythm of its central innervation. His evidence surely points strongly in that direction, but it does not necessarily follow that the same correspondence obtains in the voluntary contraction of skeletal muscles.

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[6] Garten: Zeitschr. f. Biol., 1909, lii, 555.

[7] Loc. cit., 562.

[8] Piper: Arch. f. Physiol., 1910, 209.

[9] Loc. cit., p. 216.

[10] Dittler: Pflüger's Arch., 1909, cxxx, 400.

[11] Dittler: Pflüger's Arch., 1910, cxxxi, 581.

Beritoff[12] has made some experiments with a string galvanometer on the maximum frequencies of response obtainable in nerve and muscle and on rhythm of reflex muscular responses in the frog. In discussing his results he gives an excellent review[13] of the controversy under consideration, and urges that the observed rhythm of muscle under central innervation should not be regarded as the specific rhythm of muscle exclusively, or as the rhythm of central innervation. Whether the recorded muscle rhythm in any given case is that of central innervation or is intrinsically muscular, depends, in his view, on whether or not the impulses are sent out from the center at a frequency higher or lower than the "specific rhythm" of the muscle under the conditions of the experiment. In this it seems to us he makes an important step toward a true comprehension of the problem; but we believe that in the light of certain properties of nerve and muscle recently established by Lucas and Adrian[14] the interpretation of the facts already on record together with the results of our experiments, will make the matters less confusing. To this we shall return in the discussion of results.

At all events, Buchanan's observations on the change of wavelet rhythm with change of temperature confined to the muscle is of fundamental importance. But the gap between a strychnine spasm in the decerebrate frog and the voluntary contraction of muscle in man is such as to render it highly desirable that observations similar to hers should if possible be made on human beings under conditions more like those obtaining in Piper's experiments. With this idea in mind we have performed a few experiments, using ourselves as subjects.

METHOD

Our object was to record the action currents of human muscles in voluntary contraction under as widely varying temperatures as possible and with as little variation as possible in the temperature of the central nervous system. We thought it probable that some of the small muscles of the hand lying close to the skin could be cooled appreciably by immersion in ice water and would stay cool long enough to admit of taking a record after withdrawal. But it seemed desirable first to make some test of the question whether the lowering of temperature resulting from such immersion could penetrate appreciably through the subcutaneous tissues, for it is conceivable that the circulation might be such as to keep the muscles very near normal temperature even though the skin was thoroughly chilled.

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[12] Beritoff: Zeitschr. f. Biol., 1913, lxii, 125.

[13] Loc. cit., 190–196.

[14] Lucas: Journ. Physiol., 1911, xliii, 46; Adrian and Lucas: Journ. Physiol., 1912, xliv, 114; Adrian: Journ. Physiol., 1913, xlvi, 384.

For this test a basin was filled with water maintained at approximately 7°C. and set on a stool beside the subject. A thermometer was secured with adhesive plaster so that the bulb remained in contact with the palm of the hand. The hand was then held, palm up, over the water, but not touching it, till the thermometer had come to rest and the fluctuations due to slight involuntary motions of the hand had been noted. When the temperature was steady the hand was cautiously lowered, care being taken not to permit any shift in the contact of the bulb, till the back of the hand and most of the wrist were immersed in the cold water. But no water was allowed to touch the palm. The recorded temperature at the beginning of contact with cold water was 31°C.; in four minutes it had fallen to 30°C.; in fifteen minutes it had fallen to 28.1°C. Later similar partial immersion in hot water varying between 43°C. and 47°C. raised the temperature of the thermometer from 28.1°C. to 30.9°C. in twelve minutes. From this it is evident that an easily appreciable fall of temperature can be transmitted from the surface not merely to the interior of the hand, but through it and through the skin again to the opposite surface. There is no doubt that with complete immersion of most of the forearm as well as the hand a fall of temperature can be produced in the small muscles of the hand still greater than was shown in the thermometer by this experiment. It is impossible to form any exact estimate of the degree of cooling of a muscle by this procedure, but it seems reasonable to expect from an immersion of most of the forearm in water at 6° to 7°C. for fifteen minutes a fall of more than 5°C., and perhaps a fall of more than 10°C. This should suffice to reveal a change of rhythm if conditions in human muscles are such as might be inferred from Buchanan's experiments on the frog.

The string galvanometer used in these experiments was of the Cambridge type. A full description of the electrical connections and photographic recording apparatus has already been published.[15] The arc lamp was used for illumination, and with it the simplified optical system. The 5000 ohm platinum string, hitherto designated "String C," was used throughout.

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[15] Forbes and Gregg: This Journal, xxxvii, 1915, 121–130.

The electrodes used for leading off the action current through the skin were essentially the same as those described by Piper.[16] They consisted of small glass funnels with their small ends cut off to admit rubber stoppers, and with cat bladders fastened over the large ends so that they could be filled with zinc sulphate solution. Zinc rods thrust through the rubber stoppers were thus introduced into the solution to make connection with the galvanometer. The outer surface of the bladder was soaked in salt solution when applied to the skin. When used they were secured with the bladders in contact with the appropriate skin areas by leather wristbands in which had been cut round holes a little smaller than the large end of the funnels, the wristbands being strapped around the arm as tightly as was comfortable. A saturated solution of zinc sulphate was then poured into the funnel till the rubber stopper with the zinc rod could be inserted leaving no bubble of air inside. In this way were eliminated such electrical disturbances as might result from changes in the portions of the zinc rod in contact with the fluid, which might be caused by mechanical agitation.

The muscle selected as best adapted for this investigation was the first dorsal interosseous which presents a well defined mass near the surface of the hand between the thumb and forefinger. In leading off from this muscle a small funnel electrode only about 25 mm. in diameter was applied over the belly of the muscle, while a larger electrode was placed on the back of the hand near the little finger. Both were secured in place by a single wristband with a hole for each electrode, strapped around the hand and passing through the angle between the thumb and forefinger. Contraction was produced by placing the proximal phalanx of the forefinger against the edge of a heavy table and making a strong effort to adduct the finger, which motion was opposed by the table. Another method used was to press the tip of the thumb against the side of the forefinger and exert in this way a mutual pressure.

The results with this muscle were so satisfactory that we were encouraged to repeat the experiment, using the forearm flexors which have served for the majority of electromyograms made by Piper and others, and which yield larger and somewhat more regular galvanometric excursions than the small muscle in the hand. In using this muscle group the arm was immersed in ice water to a point above the elbow. The electrodes were applied, one a large one of about 53 mm. diameter) over the flexor muscles about midway between the elbow and the wrist, the other over the tendons of these muscles at the wrist. Each was secured by a separate wristband.

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[16] Piper: Pflüger's Arch., 1909, cxxix, 149.

The experimental procedure began with washing in warm water the skin over the muscle to be studied. The object was to minimize any error that might result from comparing records taken through the skin which had not been previously moistened, with records taken through well soaked skin. Such an error could hardly influence the main question, i.e., the frequency of action currents, since the only significant alteration would probably be that of skin resistance, and such a change would affect the magnitude but not the frequency of galvanometric excursions. But it seemed desirable, none the less, to make conditions as uniform as possible, for any marked change of amplitude would be of interest.

The electrodes were then applied and a series of records was obtained with both moderate and strong contractions, the muscle being at approximately normal temperature. Then the hand was immersed in a large jar of cold water for from fifteen to twenty minutes, the temperature of the water being kept between 4°C. and 9°C. by the occasional addition of cracked ice. When only the first dorsal interosseous muscle was to be studied the arm was only immersed to a point midway between the wrist and elbow; when the forearm flexors were studied a tall jar was used which permitted immersion to a point above the elbow. Immediately after this immersion the skin was rapidly wiped dry, the electrodes were reapplied, and another series of contractions was recorded as soon as possible. In every case the records were made about four or five minutes after the hand was withdrawn from the cold water. The skin over the entire area that had been immersed felt well chilled to the touch for a considerably longer period than this, and we believe that the muscle was at the time of the records nearly as far below its normal temperature as it was at the moment of withdrawal. There also persisted a marked loss of efficiency in the cooled muscles, evidenced by the difficulty experienced in attempting to write with the chilled hand.

The hand was then immersed in water as hot as could readily be borne, i.e., between 44° and 47°C., for a period of six or eight minutes, sometimes longer. With the aid of the circulation the warming process is more rapid than the cooling process, and it seemed to us that this period sufficed to raise the temperature of the hand about as high as it was possible to raise it. Again the hand was withdrawn, the skin wiped, the electrodes reapplied and another series of records taken at a time varying from three to six minutes after withdrawal.

In two of the experiments the question of the change of body temperature was controlled by noting at frequent intervals the buccal temperature. In each case the temperature remained between 37°C. and 37.2°C. during the entire experiment. From this we may safely conclude that there was not enough change of temperature in the ganglion cells to account for any change of rhythm which might appear in the records

We encountered in one experiment a technical difficulty which it proved necessary to guard against. In one record certain oscillations occurred with more persistent regularity than is commonly seen in electromyograms; their frequency was only about 28 per second and the oscillations due to the action currents, being of higher frequency, were superimposed upon them. These oscillations were traced to the vibration of the building due to a circular saw in the machine shop two floors below. That this was possible was surprising as the galvanometer is mounted on a stand built solidly against the outside stone wall of the building. But by having the saw alternately running and still during a series of observations we were able to demonstrate the dependence on it of these oscillations.

RESULTS

The records showed with perfect uniformity a decrease in frequency together with an increase in amplitude of the excursions whenever the arm was chilled, and in most cases a somewhat less pronounced increase in frequency with a decrease in amplitude when the arm was heated. To make an accurate quantitative estimate of these changes in frequency is difficult, for, as may readily be seen by examining any of the electromyograms here reproduced, or any of those published by Piper and Garten,[17] it is possible to find in the records of any single second's duration all intermediate grades of oscillation from the largest down to barely perceptible deviations in the course of the curve. In consequence, it is sometimes hard to decide what to count. Piper has contended that a proper estimate of the true frequency of action currents should be made by counting only the "primary waves"[18] and thus avoiding confusion due to "interference."[19] In our experiments with the forearm flexors we have been careful to place the electrodes in positions corresponding closely to the points Piper designates "3" and "5,"[20] thereby eliminating as far as possible the secondary oscillations which he ascribes to "interference." In experi-

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[17] Piper: Elektrophysiologie menschlicher Muskeln, Berlin, 1912, 82–85, 97. Garten: Loc. cit., figs. 38 and 39, plate xvii.

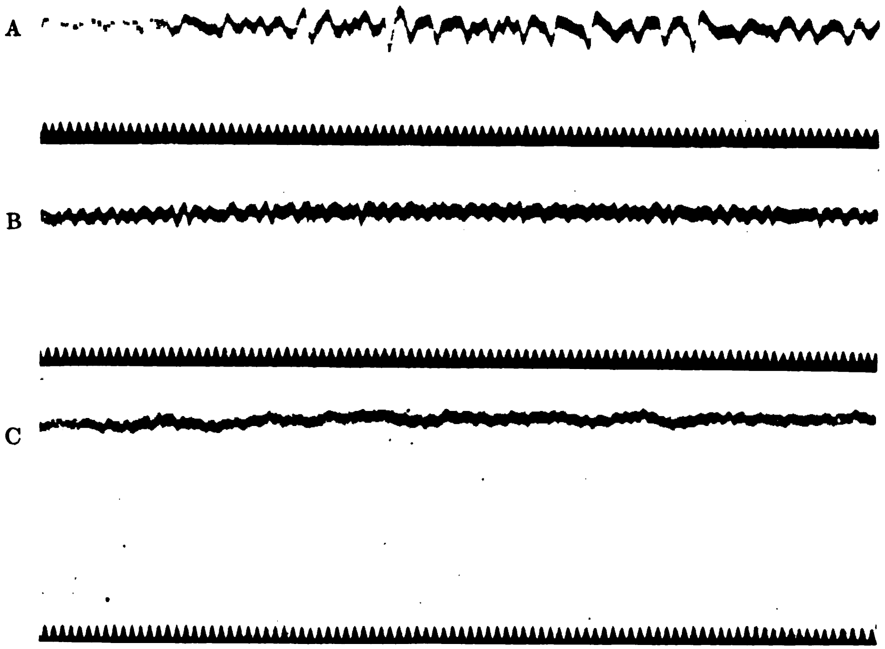

Fig. 1. Electromyograms of first dorsal interosseous muscle; adduction of forefinger against table. Experiment 3; Subject R. *A*, after fifteen minutes' immersion at 7°C. *B*, after four minutes' preliminary immersion at 40°C. *C*, after final immersion, fifteen minutes at 47°C. In all figures the upper line recording movements of the string, shows by upward excursions negativity in the electrode placed over the belly of the muscle. The lower line records time; each complete vibration = 0.01 second.

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[18] Piper: Arch. f. Physiol., 1910, 211.

[19] Piper: Pflüger's Arch., 1909, cxxix, 157, fig. 3; Elektrophysiologie, etc., 29–32.

[20] Piper: Op. cit., 22; also Pflüger's Arch., loc. cit., 150, fig. 1.

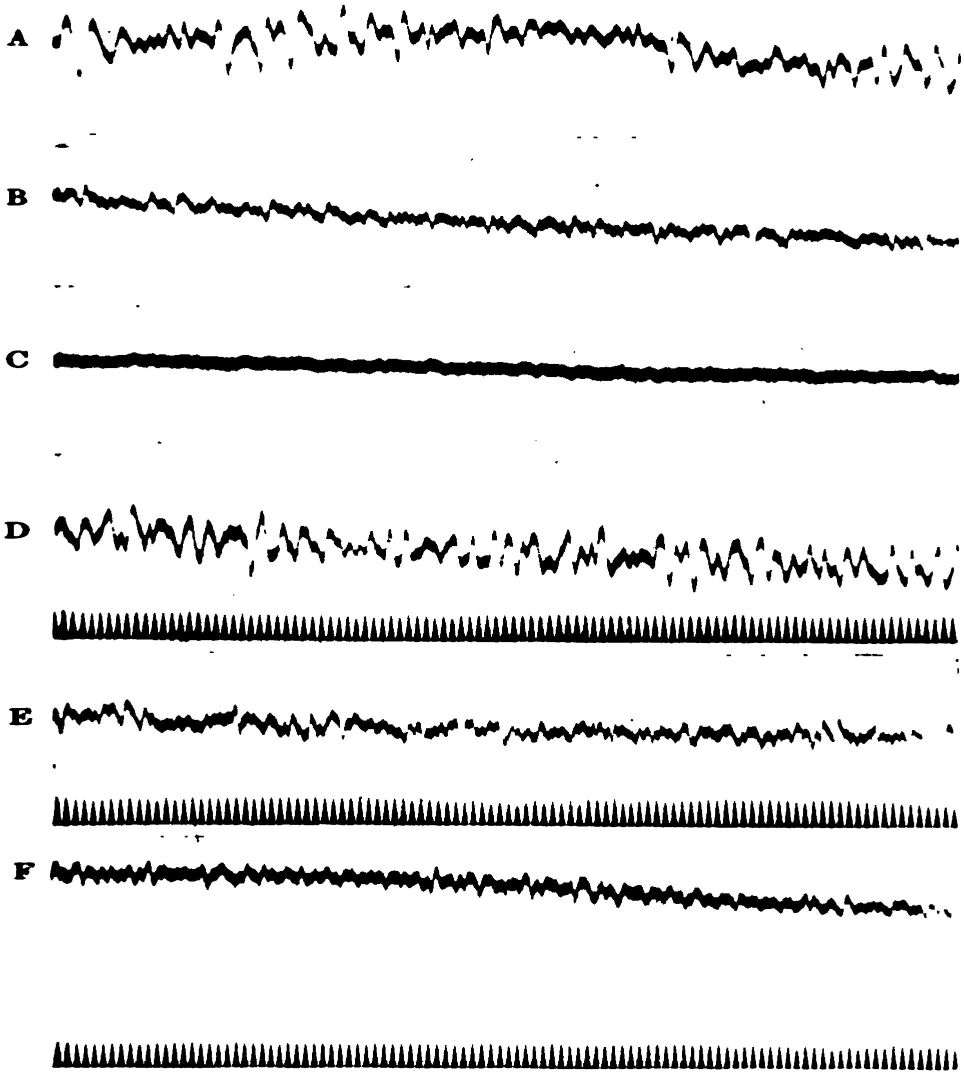

Fig. 2. Experiment 5; Subject F. First dorsal interosseous. *A*, *B* and *C* are records of adduction against table. *D*, *E* and *F* were made by pressure between thumb and forefinger (see text). *B* and *E* were taken first at normal temperature, *A* and *D* in quick succession after eighteen minutes' immersion (with a short interruption) at 4° to 5°C. *C* and *F*, after subsequent six minutes' immersion at 47°C. In this figure and in figures 3 and 4 some of the time lines have been omitted to save space, but in each experiment the speed of the film was constant in all records.

ments with the first dorsal interosseous the small size of the muscle has left little choice in the placing of the electrode intended to be at the focus of activity; and the position of the other electrode, on the back of the hand, should, as far as we can judge, serve as well as any other to render the oscillations simple in form. Yet small secondary notches appear and it is difficult to make a reliable distinction between these and the "primary waves" on whose importance Piper has insisted.

In spite of this difficulty we have found it possible to make estimates of relative frequency of oscillations, and whenever the temperature has been unchanged through two successive observations the uniformity of the estimates has justified them. The method was as follows: A length on the record corresponding to a second of time was marked off, a portion being chosen which showed a well sustained period of activity. A count was then made of major excursions, the criterion being that which seemed most nearly to make the count represent the prevailing rhythm. Then a second count was made for the same second, but this time every notch which could be detected with certainty was counted. The two figures were placed in separate

TABLE 1

	COLD		NORMAL		HOT	
	Primary	Total	Primary	Total	Primary	Total
Experiment 2; Subject F	44	80	55	112		
	45	89				
Experiment 3;† Subject R	36	69	63	104	(65)	137
	35	73	66	118		
Experiment 5; Subject F	40	74	50	107	(64)	(114)
	35	73				
	* 38	81	48	111	60	132
	41	79			63	133

† In Experiment 3 the forearm was immersed in water at 40°C. for four minutes before the first records were taken. Thus the temperature of these, listed under "Normal," was slightly higher than normal. A correspondingly high count is to be noted.

* The figures in the last two lines are from records made by mutual pressure between thumb and forefinger. All the rest are from records of pressure against the table.

columns and the records at different temperatures were compared separately on each basis. Table 1 shows a comparison of these figures taken from records of the interosseous muscle; in the columns marked "Primary" are the counts of major excursions in a second; in the column marked "Total" are the counts of all discernible oscillations. In records made after heating the arm the smallness of the excursions renders difficult a satisfactory count, and some of these figures are therefore in parenthesis to indicate that they are rougher approximations than the others. This difficulty tends to make the count too low.

The uniformity of counts made under similar temperature conditions is such as to warrant the conclusion that the difference correlated with change of temperature indicates a real change of rhythm.

TABLE 2

	KILOS	COLD		NORMAL		HOT	
		Primary	Total	Primary	Total	Primary	Total
Experiment 6; Subject F.......	20	36	46	41	62	45	70
	36	30	46*	43	62	47	67
	40					43	68
Experiment 7; Subject R.......	32	33	53				
	42					50	63
	51			47	63		

* This count was made on the record reproduced in figure 3B in which a shift in the zero current made the record overlap the time line. On this account two or three of the minor oscillations may have been missed in the count.

The forearm flexors, although extending to a greater depth below the skin and nearer to the large arteries, and consequently less easily chilled through, are in other respects more favorable for study than the smaller muscle of the hand. For, in the first place, the excursions are larger and more regularly rhythmical, and are therefore more easily counted. In the second place, it is possible to grade the intensity of contraction by means of a dynamometer. The subject gripped the instrument and, watching the pointer, maintained a steady contraction of any desired force. Thus we could compare records at different temperatures but identical strength of contraction.

Table 2 shows the results of two experiments with the forearm flexors, in the first of which the identity of strength of contraction was closely maintained.

The constancy of rhythm in a given experiment at a given temperature irrespective of the strength of contraction is strikingly illustrated here, thus supporting the statement to this effect already made by Piper.[21] This constancy serves also to emphasize the change of rhythm which occurs in this muscle group, as well as in the interosseous, with change of temperature.

Another method of estimating the action current frequency in the records taken from the forearm flexors was rendered possible by the regular rhythmicity appearing in portions of these records (see figs. 3 and 4). A large number of the most regular of these oscillations were selected and the time interval between their summits determined by careful measurement on the record. The average of those measured was then computed, and from this the average frequency of oscillations per second for the most rhythmical portions was estimated. By this method the following results were obtained

Experiment 6	Cold 36, Normal 49, Hot 50
Experiment 7	Cold 39, Normal 51, Hot 54

Here again the figures show an unmistakable correlation between temperature and frequency.

It is to be noted that in all of the figures given, the difference in frequency is far less marked between normal and hot than between normal and cold, and in some instances of the former no difference at all is revealed by the figures. This is to be expected inasmuch as the range of temperature above normal which is tolerable to the skin is far smaller than the range below normal. Especially is it probable in the case of the forearm flexors, to which the blood stream comes more directly from the large arteries, that normal temperature is too quickly re-established after withdrawal from hot water to render possible the recording of an increase in frequency with any certainty. The fact that the frequency was in no case lower and in most cases higher after heat than normal, helps to support the general conclusion which rests in the main on the comparison of normal with cold.

Although the figures we have given are of doubtful value as far as establishing a quantitative estimate of the changes, we feel that inspection of the records, fair samples of which are reproduced in figures 1 to 4, will leave no doubt that cooling the muscle slows the rhythm. And since we have no way of determining the actual fall of temperature

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[21] Piper: Arch. f. Physiol., 1910, 213.

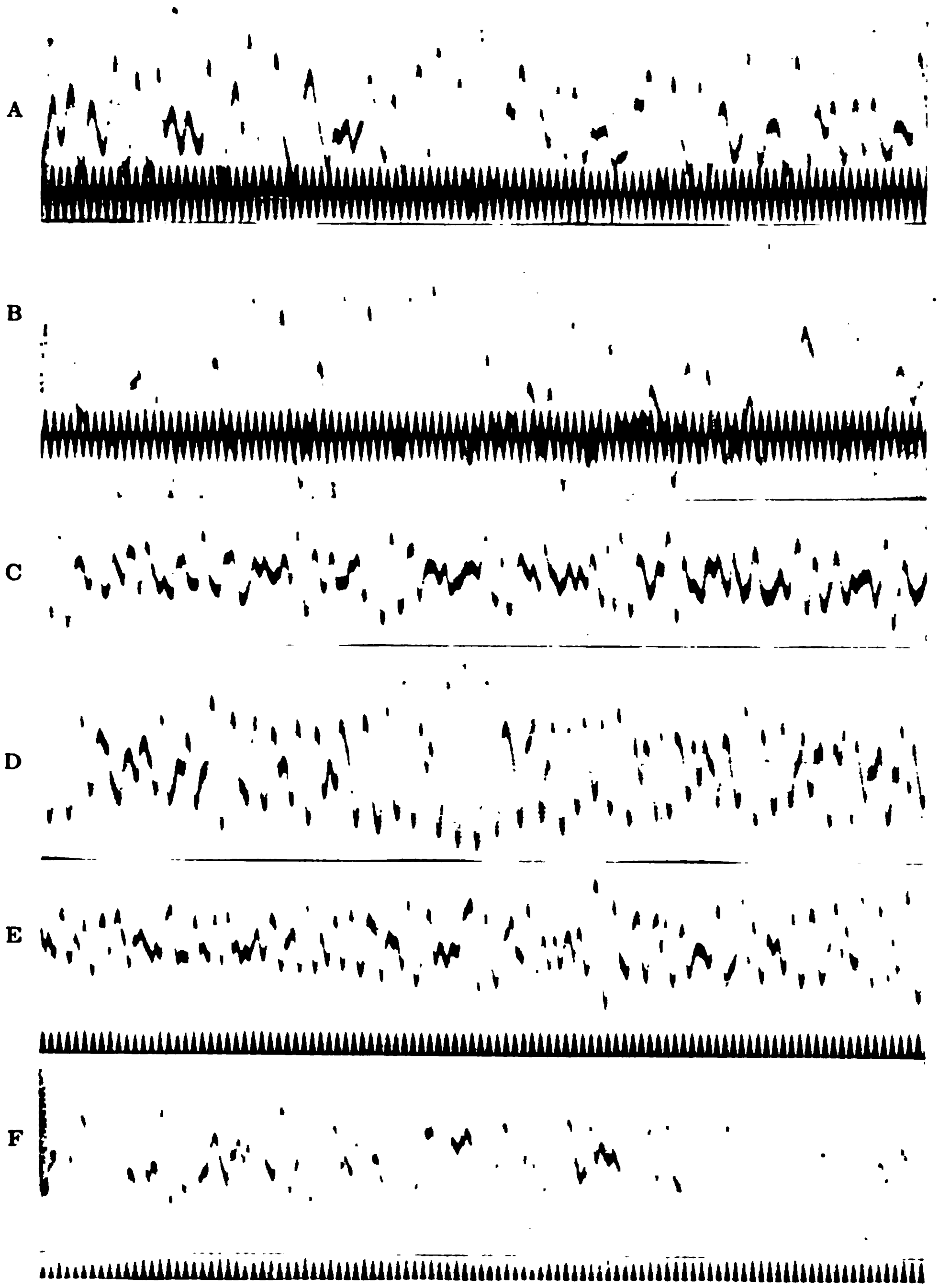

Fig. 3. Experiment 6; Subject F. Forearm flexors. *A* and *B* cold, after nineteen minutes' (interrupted) immersion, 4° to 9°C. *C* and *D* normal temperature. *E* and *F* hot, eight minutes' immersion 44° to 46°C. Strength of contraction in *A*, *C* and *E*, 20 kilos; in *B*, *D* and *F*, 36 kilos.

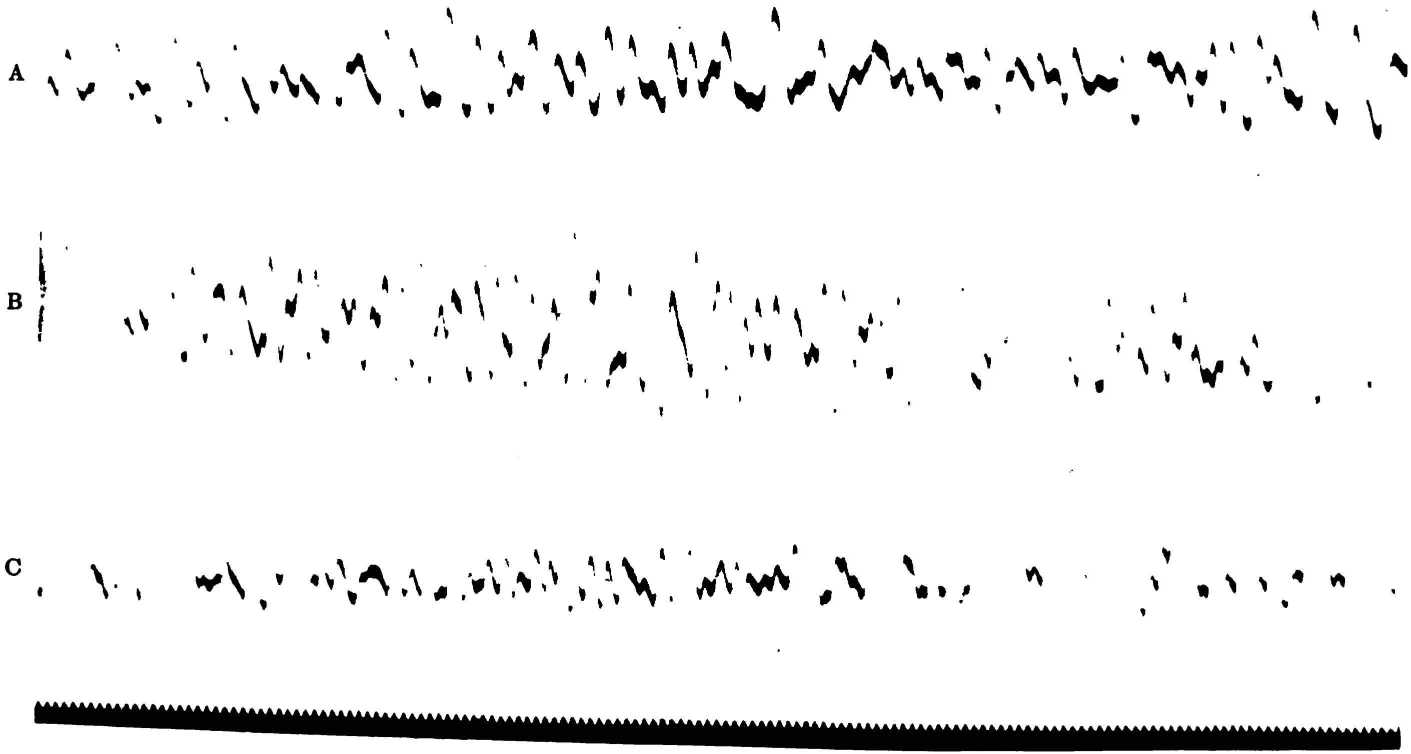

Fig. 4. Experiment 7; Subject R. Forearm flexors. *A*, cold, immersion fifteen minutes, 6°C., contraction 32 kilos; *B*, normal temperature, contraction 51 kilos; *C*, hot, immersion six minutes, 45° to 46°C., contraction 42 kilos.

in the muscles, an exact determination of the decrease in frequency would be of little value. The important fact is that an unmistakable decrease in frequency does occur when the muscle is cooled.

DISCUSSION

It seems to us to follow from the above results that the rhythm of action currents appearing in the electromyogram of human voluntary contraction is no direct index of the rhythm of central innervation involved in the act. The change of rhythm attending change of temperature would be inexplicable on any such basis. For why should a change in the temperature of the muscle cause a change in the frequency of discharge of impulses from the ganglion cells whose temperature remains constant? This might conceivably be if afferent impulses coming from the chilled muscle so modified the nerve center as to alter its frequency of discharge; but this assumption is far-fetched and invokes nervous influences to which we know of no analogy. And since all the known phenomena, so far as we are aware, can be explained in terms of definitely known properties of nerve and muscle, without any such speculative assumption, it seems unnecessary to attach much weight to it. We agree, then, with Buchanan that the rhythm in the muscle does not follow the rhythm of motor nerve impulses, but depends rather on the condition of the muscle itself.

We now come to the task of reconciling this view with the fact pointed out by Piper and Beritoff,[22] that a frog's muscle can follow with separate action currents the rhythm of nerve impulses excited by induction shocks even up to a frequency of 250 or 300 per second. In man we have a higher possible frequency to reckon with, for V. Stern[23] has found by auscultating a muscle in a rabbit that a limiting frequency of 365 per second is possible in the mammalian muscle under like conditions of stimulation; and even without this observation we should expect a higher frequency under the temperature conditions prevailing in man than in the nerve-muscle preparation of the laboratory. We have here an apparent paradox; the muscle can respond separately to more than 300 nerve impulses per second, but when played on by the stream of impulses coming from the ganglion cells it responds with frequencies which may be evenly graded from 30 to 50 or more per second according to its own temperature. Sources of stimulation

[22] Piper: Arch. f. Physiol., 1910, 209; Beritoff: Loc. cit., 170.

[23] Stern: Pflüger's Arch., 1900, lxxxii, 39 (see Beritoff: Loc. cit., 171).

must be available at such frequency that the muscle can be excited at several frequencies between 30 and 50 per second. This requires the existence of such sources of stimulation at a frequency probably not less than 300 or 400 a second.

We are confronted here by the uncertainty as to whether we are dealing with propagated disturbances sweeping over the muscle "in a volley," i.e., simultaneously in all the fibers, or whether different groups of fibers respond in alternation or rotation.[24] In the latter case the rhythm of the individual fiber would be much slower than the rhythm appearing in the record. Piper's careful analysis[25] of the waves in his electromyograms, based on a study of artificially induced isolated waves, leads to the view that the rhythm appearing in the major oscillations is the actual rhythm of response in the individual muscle fiber. The fact that the frequency does not change with increased strength of contraction is in favor of this view. Inspection of a typical record suggests that where the curve is irregular it is due to certain fiber groups getting "out of step;" but where the oscillations are large and smoothly rhythmical, each double vibration closely resembling in size and time relations the record of a single response of the whole muscle,[26] it is highly probable that the majority at least of the fibers are responding in unison. If this is so, then the least number of nerve impulses in a given time would exceed the least common multiple of the various possible numbers of muscular responses which could, by changing the temperature, be made to occur in the same time with perfectly regular rhythm. Individual time measurements of the most regular oscillations in our records have been examined with a view to detecting any grouping which would on this basis suggest a nerve rhythm. Although it is possible to find practically all gradations in the intervals, yet with one subject (experiment 6) they seemed to be distinctly grouped at multiples of an interval which would indicate a nerve impulse frequency of 390 per second; with the other subject (experiment 7) a less distinct grouping was found such as would indicate a nerve impulse frequency of 260 per second. The intermediate values, especially in the latter case, are sufficiently numerous to render a higher frequency than the last mentioned more probable. A conservative estimate would probably place 300 per second as the lower limit to the frequency of voluntary innervation. If this be the case, why does not the muscle respond at the maximum frequency possible, which Beritoff has found to be 250 per second in frog's muscle and is probably higher in human muscle?

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[24] Cf. Barbour and Stiles: Amer. Phys. Education Rev., Feb., 1912.

[25] Piper: Pflüger's Arch., 1909, cxxix, 150–168.

[26] See Piper: Elektrophysiologie, etc., 97, figs. 33 and 34.

The answer to this question may be found by examining the properties of nerve and muscle demonstrated by Lucas and Adrian.[27] In the first place, in considering the nature of the central stimulus, while we agree with Buchanan[28] that we have in these experiments no proof that it is discontinuous, we have good evidence from other sources that it is discontinuous or rhythmic and not in the nature of a steady flow of energy. Garten[29] has shown that stimulation with a constant current produces rhythmic electrical responses in a nerve. Moreover, the well established property of refractory period in nerve necessitates intermittent response. We must, then, regard the central stimulus as a series of separate impulses of unknown frequency; and our evidence seems to show that this cannot be less than 300 to 400 a second.

It is also well established that the refractory period in nerve and muscle has a "relative" as well as an "absolute" stage. Following the period in which no stimulus will excite there is a time during which the threshold falls gradually from infinity to its normal value.[30] During this "relative refractory period" of lowered excitability the magnitude of response is also subnormal. This has been shown by two wholly distinct criteria. First, Lucas[31] has shown that in nerve the action current resulting from a stimulus applied during this time is smaller than normal. Second, Lucas and Adrian[32] have both shown that a nerve impulse evoked during the relative refractory period is extinguished in a region of impaired conductivity through which a full-sized disturbance would pass. The recovery of excitability and the recovery of the magnitude of obtainable response proceed together during this time. Adrian[33] has also shown that at all times the magnitude of response in the conducting unit of nerve depends only on the local condition of the tissue, including under this head the stage of recovery during the relative refractory period, and is independent of the strength of stimulus.

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[27] See references on p. 231, footnote 14; also Lucas, Proc. Roy. Soc., 1912, 85B, 495; and Adrian: Journ. Physiol., 1914, xlvii, 460.

[28] Buchanan: Quart. Journ. Exper. Physiol., 1908, i, 231.

[29] Garten: Loc. cit., 557.

[30] Adrian and Lucas: Journ. Physiol., 1912, xliv, 114.

[31] Lucas: Journ. Physiol., 1911, xliii, 51 and 77.

[32] Lucas: Loc. cit., 74; Adrian: Journ. Physiol., 1913, xlvi, 384.

[33] Adrian: Journ. Physiol., 1913, xlvi, 412.

With these facts in mind we may consider the question why the muscle exhibits in voluntary contraction only 50 responses per second when it is capable of 300 responses per second and is subjected to a still larger number of nerve impulses. The properties of these tissues just mentioned reveal to us the error in assuming for each tissue a "specific rhythm." For if a tissue be subjected to a continuous stimulus, the frequency with which it responds will depend on the intensity of the stimulus, for on this will depend the stage of recovery from one response at which the stimulus will become adequate and produce the next. As has already been pointed out by one of us[34] this variability of rhythm is well illustrated in the familiar class-room experiment on the "stannius heart," and the application of the principle to nerve has been well stated by Adrian.[35]

On this principle let us analyse the course of events in the neuromuscular system. Adrian and Lucas[36] found for frog's nerve at 15°C. an absolute refractory period of about 2σ and a total refractory period (including the "relative stage") of about 12σ. For frog's muscle[37] (probably at a similar temperature) they found an absolute refractory period of about 10σ and a total refractory period of about 70σ. They cite Bazett[38] as having shown in muscle a change of refractory period in a ratio of about 1: 3 for a temperature change of 10°C. If the times are of the same order in human and in amphibian tissues, we may expect an 8- or 10-fold reduction of the refractory periods given above, in human tissues. Thus the relative refractory period for nerve would last roughly from 0.25σ to 1.5σ after an effective stimulus, and for muscle from about 1.2σ to 10σ. The observations of Garten[39] on the mammalian nerve and muscle make it probable that these figures are too small. For the purposes of our analysis let us assume that the relative refractory period in human nerve begins 0.8σ and ends 7σ after the beginning of a previous response, and that in muscle it begins 3σ and ends 25σ after an effective stimulus.

If the nerve impulse when of subnormal magnitude serves as a weaker stimulus to the muscle fiber than it does when of normal magnitude, we have all the conditions necessary for a satisfactory explanation of the facts. Let us consider the stimulating value of the nerve impulse to the muscle as represented in an arbitrary scale of units in which we can also express quantitatively the threshold of the muscle. We can then plot a curve representing the magnitude the nerve impulse would have if evoked at each instant during the relative refractory period, i.e., during the recovery of the nerve to normal; and the magnitude will be expressed in terms of its stimulating value to the muscle. On the same system of coordinates we can plot a second curve representing the return of the threshold of the muscle fiber from infinity to normal during its relative refractory period; and the threshold will be expressed in terms of the requisite stimulating value of the nerve impulse. The

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[34] Forbes and Gregg: This Journal, 1915, xxxix, 231.

[35] Adrian: Loc. cit., 385–386.

[36] Adrian and Lucas: Loc. cit., 114.

[37] Loc. cit., 89.

[38] Bazett: Journ. Physiol., 1908, xxxvi, 426.

[39] Garten: Loc. cit., 543–552.

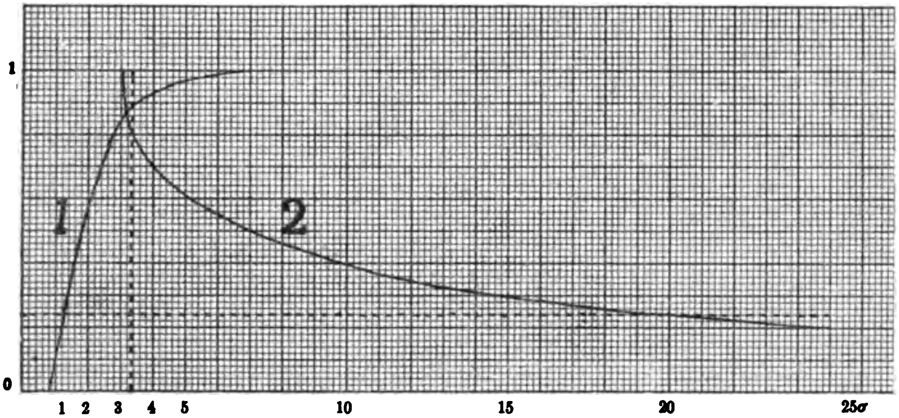

Fig. 5. Abscissae, time in σ since previous adequate stimulus. Ordinates, stimulating value of nerve impulse (curve 1), threshold of muscle (curve 2), see text.

curve of nerve recovery will have in a general way the shape indicated in curve 1, figure 5; the curve of muscle threshold will be in a general way like curve 2.

We can now examine the course of events when the muscle is made to respond 300 times a second to as many induction shocks applied to the motor nerve, which Beritoff and Piper have both shown is possible. Each stimulus comes 3.3σ after its predecessor and each nerve impulse after the first will have the magnitude indicated on curve 1 at this point of time. If the muscle has responded to the previous stimulus its threshold will have at this instant the value indicated on curve 2. If the value indicated by curve 1 is higher at this instant than the value indicated by curve 2 the muscle will be again excited. In the particular case represented by these curves it is clear that the muscle will be excited again at the stated time 3.3σ after the previous stimulus. The limit of frequency with which the muscle can respond to stimulation through its nerve depends on the time at which the curves cross. The validity of the principle invoked does not depend on the accuracy of these particular curves in expressing all the quantities. The procedure is merely a method of schematically representing established principles which determine the maximum frequency of response in a muscle stimulated through its nerve.

The next step is to trace the course of events in sustained voluntary contraction of muscle. Here, if our reasoning is correct, we are dealing with nerve impulses of a higher frequency than those just considered; each impulse occurs earlier in the relative refractory period following its predecessor and is consequently smaller and acts as a weaker stimulus to the muscle. After the muscle has once responded its threshold returns to normal so slowly that if the nerve impulses follow each other close enough several may pass before another suffices to excite the muscle; for the muscular threshold remains above the stimulating value of the weaker impulses throughout a larger part of its relative refractory period than in the case of the less frequent and stronger impulses. In the particular case illustrated by the curves it is only necessary to assume that the nerve impulses have a frequency of 800 per second in order that we may have all the conditions for a 50 per second rhythm in the muscle. This may be seen by reference to figure 5. Every nerve impulse following its predecessor at an interval of 1.25σ (i.e., 1/800 sec.) has the stimulating value required to excite the muscle 20σ after a preceding response, as is indicated in the figure by the horizontal dotted line. All the nerve impulses falling in the earlier part of the muscle's refractory period are ineffective.

Again we must note that the validity of this analysis does not rest on the correctness of the quantities we have assumed. The rhythm of 800 per second happens to be that which, according to the curves as we have drawn them, would condition a muscle rhythm of 50 per second. If the curves of recovery for nerve and muscle could be accurately determined and plotted, the requisite nerve impulse frequency might prove quite different; but the principle would apply just the same.

Thus is explained how a higher frequency of nerve impulses can cause a lower frequency of muscle responses. The application of this principle has been experimentally demonstrated by both Beritoff and

Buchanan. Beritoff[40] found that whereas the muscle kept pace with the rhythm of nerve stimulation up to 250 per second; with higher frequency of stimulation (300 per second) the frequency of muscular response fell to 150 per second, and in portions of the record, to 100 per second. Buchanan[41] found that stimulation of a motor nerve or of a curarized muscle with frequencies of 1000 per second produced in the muscle frequencies of response between 50 and 100 per second. When the frequency of stimuli was increased above 1000 no change occurred in the resulting muscle rhythm, and it is probable that these stimuli were virtually continuous even to the nerve, i.e., that the nerve was unable to follow the rhythm of stimulation, but responded in a rhythm dependent on its own refractory period.

We are now in a position to reinforce our previous argument for a high frequency of nerve impulses. We showed experimentally that the muscular rhythm of 50 per second was not the rhythm of the motor nerve, and that judging from the frequencies obtainable in muscle, the nerve rhythm must be much more rapid than this, probably not less than 300 per second. Now in the light of our analysis we can state that the nerve frequency cannot be less than 300 per second, for if it were the muscle would be able to follow it; and this it obviously does not.

Another fact noted by Beritoff,[42] harmonizing with the view here set forth, is that when the frequency of nerve stimulation is raised above 100 per second the muscle rhythm fails to follow regularly unless the stimuli are somewhat above threshold value. At this frequency he was already encroaching on the relative refractory period of the nerve, which under like conditions Adrian and Lucas[43] found to end about 12σ after the previous stimulus. With frequencies between 200 and 250 per second much stronger stimuli were needed to make the muscle follow the rhythm imposed, for now each stimulus found the excitability of the nerve far below normal. Buchanan[44] reports a similar observation. V. Stern[45] also found on ausculation that in the rabbit with stimuli of more than the critical frequency of 365 per second the tone fell to an octave below the stimulation pitch if the stimuli were strong, but with weak stimuli it fell two or even three octaves.

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[40] Loc. cit., 170, fig. 26.

[41] Buchanan: Journ. Physiol., 1901, xxvii, 125.

[42] Loc. cit., 166.

[43] Loc. cit., 114.

[44] Buchanan: Quart. Journ. Exper. Physiol., 1908, i, 236.

[45] Loc. cit.

One important conclusion should be drawn from this discussion, that it is not permissible to assume that any tissue has one "specific rhythm" peculiar to itself.[46] The rhythm or response obtainable from a tissue is a resultant of the curve of recovering excitability and the strength of stimuli employed. In the case of muscle excited through its nerve we have two refractory periods to consider, that of the muscle itself and that of the nerve, and also the lowered stimulating value of the impulse traversing the nerve during its relative refractory period. Thus in the case of voluntary contraction we cannot say that the rhythm of muscular response is wholly of peripheral origin because it depends on peripheral conditions, for it depends also on central conditions and is therefore of compound origin, a resultant of several causes.

Piper[47] in support of his view that the rhythm of central innervation is 50 per second has presented the following evidence. Although the muscular rhythm is constant when the strength of contraction is changed, yet it is possible to reduce the frequency of recorded waves to one-half "by continuing a strong voluntary contraction to the point of complete fatigue." This, he argues, is to be explained by the failure of the fatigued central nervous system to send out the usual number of impulses. This interpretation follows, in his view, from the fact that the individual action currents recorded still show their normal amplitude and duration, therefore the muscle is not fatigued and the change must be associated with the nerve center.

These facts are quite as easily explained in another way which does not conflict with the evidence pointing to a higher nerve impulse frequency. Lucas[48] has shown that in a fatigued nerve-muscle preparation the resistance to the passage of a propagated disturbance increases at the neuromuscular junction. The result of this in the case of voluntary contraction, maintained, as we contend, by subnormal nerve impulses of high frequency, would be to cut down still further the stimulating value of the impulses at the point where they act on the muscle fibers, and consequently to delay until nearer the end of their relative refractory period the time at which they would respond. Thus, without change in the nerve impulse frequency, the mere raising of resistance in the neuromuscular junction would slow down the rhythm of muscular response.

[46] See Garten: Loc. cit., 535.

[47] Piper: Arch. f. Physiol., 1910, 214.

[48] Lucas: Journ. Physiol., 1911, xliii, 76.

The fact shown by Piper and confirmed in our records that the rhythm of voluntary muscular response in absence of fatigue is independent of the strength of contraction brings up an interesting question as to the mechanism of gradation. One of us[49] in a previous paper has proposed as a basis of sensory gradation the rhythm of afferent impulses, which could easily be conditioned by the intensity of peripheral stimulation on the same principle of relative refractory period which we have just been considering. That a similar gradation of muscular contraction can depend on the frequency of muscular response seems to be disproved by the above observations. So far as we can see, unless we reject the "all-or-none" view of muscle contraction, so well supported by Lucas,[50] we are forced to the view that in voluntary contraction gradation must be conditioned by gradation in the number of muscle fibers in action at a given moment, the doctrine of fractional activity, as Stiles[51] has termed it.

It would be interesting to form an estimate, if possible, of the actual frequency of nerve impulses involved in voluntary muscular contractions. We have furnished evidence which indicates that it is probably not less than 300 or 400 per second. We have shown that from the refractory periods measured in frog's nerves and the temperature coefficient determined for them, we can infer that the absolute refractory period in mammalian nerves at their normal temperature may be as short as 0.2σ, which would make possible an extreme frequency of 5000 per second. Between these limits we have little to guide us in estimating the probable frequency. Garten,[52] stimulating mammalian nerves at their normal temperature with a constant current, obtained action currents showing frequencies between 300 and 500 per second. These values do not necessarily show the highest possible frequency, since the intensity of the stimulus may have been so low as only to excite the nerve toward the end of its relative refractory period.

Whatever the frequency of impulses in the motor nerve under natural conditions of central discharge, it probably bears some relation to the refractory period of the nerve fiber. We feel that this consideration tends in a way to support the general conclusion that the nerve impulse frequency is much higher than that of the action currents recorded in the muscle. Otherwise the wonderfully short refractory period of nerve would appear a meaningless and useless property. That a nerve fiber can recover its ability to respond with such great rapidity is one of its most remarkable properties and one which perhaps more than any other marks its high development in the evolutionary process. To suppose, then, that in the ordinary performance of its function this ability should be wasted, and that although capable of transmitting several hundred impulses per second the nerve fiber is only called on to transmit fifty, implies a lack of economy quite out of keeping with the usual degree of adaptation found in the functions of living tissues. It might be argued that the high frequency of nerve impulses is wasted if the muscle only responds to a small proportion of these; but a large excess of available impulses may serve a useful purpose by providing in some way a greater variability in the timing and coordination of muscular response.

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[49] Forbes and Gregg: This Journal, 1915, xxxix, 229–232.

[50] Lucas: Journ. Physiol., 1905, xxxiii, 125; 1909, xxxviii, 113.

[51] Stiles: Amer. Phys. Education Rev., 1910, xv, 1; Barbour and Stiles: Loc. cit.

[52] Garten: Loc. cit., 546 and 554.

In a previous paper one of us[53] has mentioned reasons for suspecting that the central elements involved in reflexes may have much shorter refractory periods than even the nerve fibers. Should this prove to be a general and essential property of the central nervous mechanism, it might be that the activity of this mechanism whenever involved in the causation of sustained muscular contraction is even more rapidly rhythmical than the impulses in the motor nerve fiber. This mechanism may bear the same relation to the nerve fiber that the nerve fiber does to the muscle, in that it supplies to the nerve fiber a series of stimuli of such high frequency that they amount to a continuous stimulus as far as the nerve fiber is concerned, and the nerve fiber responds with a frequency determined by its own relative refractory period. But this suggestion is too highly speculative to merit more than passing mention.

The "waves" which Buchanan[54] has found in her records of strychnine spasm in the decerebrate frog, occurring with frequencies between 3 and 14 per second, and which she showed to be associated with the condition of the nerve centers, obviously cannot be regarded as indicating the frequency of individual nerve impulses. Such slow rhythms as these should be classed, together with other slow rhythms such as are seen in clonus, the scratch reflex and others noted myographically,[55] as group rhythms. Each beat in such a rhythm must represent a large group or series of nerve impulses discharged from the center at a far higher frequency, and followed by a lull or cessation of activity. Many such rhythmic tendencies are evident in the functioning of nerve centers, but their essential nature has yet to be explained.

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[53] Forbes and Gregg: Loc. cit., 221.

[54] Buchanan: Journ. Physiol., 1901, xxvii, Plates vii, viii and ix; also Quart. Journ. Exper. Physiol., 1908, i, 215, etc.

[55] See Sherrington: Quart. Journ. Exper. Physiol., 1913, vi, 284; Proc. Roy. Soc., 1913, 86B, 233; Graham Brown: Proc. Roy. Soc., 1912, 85B, 278; Forbes: Proc. Roy. Soc., 1912, 85B, 289.

One striking feature of our records, which has already been mentioned, is the increased amplitude in the galvanometer excursions on cooling the muscle, and their decreased amplitude on heating. This is in part due to the lag of the string, which being of platinum has considerable inertia. This lag or failure of the string to follow electromotive changes with precision, distorts the curve more in the case of rapid oscillations than in the case of slow ones. The result is a slight apparent decrease in magnitude as the oscillations become more rapid. But the change in action current frequency is so small and the change in amplitude of the excursions so great that we feel little doubt that there is a true increase in the strength of individual action currents when the muscle is cooled.

In accordance with our interpretation of the phenomena such an increase in the magnitude of individual responses might be explained in one of two ways. One explanation depends on the conceivable separability of excitability and magnitude of electrical response in the process of recovery from the refractory period. If in a cool muscle the excitability is longer delayed in its return to normal than is the possible magnitude of action current resulting from an adequate second stimulus, then the action currents, under such conditions of nerve stimulation as we have pictured, will be increased in magnitude. For the stimuli delivered by the nerve will not be effective on account of the lowered excitability, until a stage of recovery at which the magnitude of response will surpass that found at the higher temperature.

Such an explanation is most unlikely since Adrian[56] has shown that in the case of nerve the possible magnitude of disturbance, as judged by the functional criterion of its ability to pass through a region in which it undergoes a decrement, recovers simultaneously with excitability, after the passage of a previous disturbance. That the action current is a true measure of the functional magnitude of a propagated disturbance is not proved, but all evidence points strongly to that view.[57] Again, Adrian proved his point for nerve and not for muscle, but for muscle to differ from nerve in the separability of excitability from magnitude of response in the recovery process would imply a fundamental difference between the tissues with respect to a property in which all evidence points to their essential similarity.[58]

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[56] Adrian: Journ. Physiol., 1916, l, 345.

[57] Forbes, McIntosh and Sefton: This Journal, 1916, xl, 503.

Another explanation which invokes no such improbable property in muscle as the separation of excitability and magnitude of response in the recovery process, depends on the cooling of the distal portions of the nerve fibers which innervate the cooled muscle. Impulses traversing these fibers in their relative refractory period are subnormal, and on passing from a warm to a cool portion of the nerve they enter a region where the refractory period is prolonged, and are still further reduced in magnitude. From this it follows that they will not excite the muscle till still later in its relative refractory period than would be the case if the nerve fibers were warm throughout their course. The muscle excited later in its recovery stage, will yield a larger response. Thus may readily be explained the increased magnitude of action currents in the cooled muscle without the need of any improbable assumption.

SUMMARY

1. By local immersion in cold water it is possible to reduce the frequency of the rhythmic action currents in the muscles of the hand or forearm during voluntary contraction, while the body temperature, recorded by mouth, remains constant. Immersion in hot water makes the rhythm more rapid, but the change is less marked than in the case of cold water, as is to be expected from the fact that a bigger change of temperature is possible below than above normal.
2. This evidence shows that the rhythm observed in muscle response (usually about 50 per second) is not the rhythm of motor nerve impulses, but is dependent, as Buchanan maintains, on the condition of the muscle.
3. A careful study of the possible gradations of muscular rhythm, together with a consideration of other known facts concerning the refractory period in nerve, leads to the conclusion that the actual frequency of motor nerve impulses involved in evoking voluntary contraction must lie somewhere between 300 and 5000 per second.
4. Previous objections to the view that the nerve impulse frequency is higher than that appearing in the muscular response are answered by an analysis of the relation between the "relative refractory periods" of nerve and muscle.

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[58] See Lucas: Proc. Roy. Soc., 1912, 85B, 495.

5. We have confirmed Piper's observation that the action current rhythm in muscle is independent of strength of contraction.

6. Besides the decrease in frequency following a fall of temperature there is an increase in amplitude of galvanometric excursions, denoting an increase in the strength of the individual action currents. This can most easily be explained as a result of the cooling of the distal portions of the nerve fibers, the explanation depending on the various factors involved in the relative refractory periods of the nerve and muscle fibers.

BILE PIGMENT METABOLISM

III. Bile Pigment Output and Blood Feeding

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When it was shown that the bile pigment secretion in the dog can be influenced at will by changes in diet, it became imperative to try various substances rich in the pyrrol nucleus. This paper deals with the influence of blood feeding on the bile pigment elimination in the dog.

In earlier publications we have shown that carbohydrates tend to increase considerably the secretion of bile pigments in dogs with bile fistulae (1). On a strict meat diet the bile pigment curve is at its lowest level, but with a sharp transition to a diet rich in carbohydrates, a rise of 50 to 100 per cent in bile pigment output may be noted. We believe sufficient experimental data are at hand to prove this point beyond a doubt, but the explanation of this peculiar and important reaction is not clear. We hope to report experiments in the near future which will throw some light on this important point.

It is very tempting to assume that the liver can construct certain pigments regardless of more or less blood destruction or that it can assemble more or less completely some parent pigment complex which may be used for one purpose or another as needed in the body economy. This is a stimulating possibility to be kept in mind, and is responsible for much of this work to be reported. Whether this hypothesis can be established beyond a reasonable doubt remains to be seen.

Our first paper (2) reviewed the work of other investigators in this field, and pointed out some of the many difficulties met with in work with these bile fistula dogs. It was shown that under very uniform conditions of health and routine collection the output of bile pigments becomes quite constant for days, weeks and months. The mean output per six hour period is 1 mgm. per pound body weight, or very slightly below this. There are hourly and at times daily variations which can not be explained in the light of our present knowledge. For this reason experiments must be repeated time and again with extremely careful control observations. This point cannot be too strongly emphasized, as has been done by Stadelmann (3).

The various experimental procedures employed in this work have been carefully described by us in earlier papers (2), and will not be reviewed here. A new method which is rapid, simple and accurate for the quantitative estimation of bile pigments in whole bile was described in detail. There are no departures from these published methods, unless note is made to that effect. It will be noted that several of the bile fistula dogs are marked with a splenectomy. There are many interesting points to come up for presentation in another paper dealing with splenectomy and bile fistulae, but in these feeding experiments the absence of the spleen has no effect.

TABLE A

Fresh sheep blood feeding and bile pigment secretion

Dog 15–22. Simple bile fistula

DATE	BILE											REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Weight	Mixed diet
1916											*lbs.*	
March 15	35	27	30		92	13.3	8.2	10.7		32.2	31.0	
March 16	32	33*	23	27	83	11.6	13.4*	9.4	10.3	33.1	31.5	300 cc. sheep blood
March 17	33	32	27		92	12.0	10.8	9.1		31.9	30.8	
March 18					52					32.8	31.5	
March 20	25	24	31		80	8.9	9.6	10.4		28.9	31.5	Hemoglobin 116 per cent

Dog 15–27. Bile fistula and splenectomy

DATE	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Weight	REMARKS
March 7	18	23	23		64	8.4	9.4	7.9		25.7	25.5	Hemoglobin 82 per cent
March 8	17	17	22		56	6.5	7.6	8.4		22.5	26.0	
March 9	19	26*	24	19	69	7.7	8.2*	8.1	6.8	23.1	26.0	500 cc. sheep blood
March 10	9	17	24		50	7.3	7.6	7.6		22.5	26.3	
March 11					42					20.8	26.5	

* Fresh sheep blood given through stomach tube at the beginning of the third hour.

TABLE B

Fresh pig blood feeding and bile pigment secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 24	33	35	21		89	12.0	11.0	6.2		29.2	31.5	
March 25					94					28.8	32.0	Hemoglobin 119 per cent
March 27	22	26	25		73	10.4	11.6	8.5		30.5	31.5	
March 28	34	26*	26	25	77	10.0	7.6*	10.5	9.0	27.1	31.5	350 cc. pig blood
March 29	26	29	29		84	7.6	10.4	9.1		27.1	32.3	Stool contains stercobilin
Dog 16-10. Bile fistula and splenectomy												
March 24	29	36	21		86	12.4	15.8	10.9		39.1	32.0	Stools contain stercobilin
March 25					56					37.6	31.5	
March 27	17	17	28		62	13.0	8.8	15.2		37.0	31.0	Hemoglobin 97 per cent
March 28	20	21*	26	28	75	14.1	12.2*	13.1	14.0	39.3	31.5	350 cc. pig blood
March 29	22	31	23		76	13.8	13.6	14.6		42.0	32.0	Stools contain no stercobilin

* Fresh pig's blood given through a stomach tube at the beginning of the third hour.

TABLE C

Fresh ox blood feeding and bile pigment secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1-2 hrs	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 8	25	28	28		81	8.5	8.8	10 0		27.3	30.8	Hemoglobin 112 per cent
March 9	28	37*	21	28	86	9.4	9.7*	6.9	10.0	26.6	31.5	400 cc. ox blood
March 10	24	25	25		74	9.8	9.0	8.5		27.3	31.3	
March 11					64					26.4	31.5	
March 13	20	25	19		64	9.9	10.8	7.6		28.3	31.3	Hemoglobin 114 per cent
Dog 16–41. Bile fistula and splenectomy												
March 10	13	15	30		58	10.2	7.7	12.8		30.7	37.0	
March 11					58					32.8	37.3	
March 13	17	27	31		75	9.2	10.3	9.8		29.3	37.0	Hemoglobin 81 per cent
March 14	34	27*	40	46	113	11.5	10.3*	9.2	9.6	29.1	37.3	500 cc. ox blood
March 15	48	36	27		111	10.5	11.2	11.5		33.2	37.8	

* Fresh ox blood given through stomach tube at the beginning of the third hour.

TABLE D

Fresh dog blood feeding and bile pigment secretion

Dog 16–10. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 7	21	27	24		72	11.3	12.2	9.2		32.7	31.3	Hemoglobin 90 per cent
March 8	25	22	22		69	10.8	10.4	10.9		32.1	31.8	
March 9	21	17*	18	20	55	16.5	13.0*	13.8	13.6	40.4	32.0	500 cc. dog blood
March 10	23	21	25		69	14.0	13.4	13.5		40.9	31.8	
March 11					46					36.2	31.8	
Feb. 28	13	23	23		59	10.2	10.4	10.4		31.0	31.0	Hemoglobin 88 per cent
Feb. 29	21	19*	19	21	59	13.4	11.5*	10.8	9.4	31.7	30.5	200 cc. dog blood
March 1	23	19	20		62	15.8	12.8	13.6		42.2	30.5	
March 2	18	22	24		64	4.5	15.2	14.0		33.7	31.0	
March 3	23	25	22		70	15.8	14.6	15.4		45.8	31.8	

* Fresh defibrinated dog blood given through stomach tube at the beginning of the third hour.

TABLE E

Hemoglobin feeding and bile pigment secretion

Dog 16–104. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 1	25	19	19		63	10.2	8.9	8.9		28.0	33.0	
March 2	24	23*	29	27	79	11.3	10.3*	11.7	10.3	32.3	32.8	150 cc. laked red cells
March 3	23	23	18		64	8.2	10.9	8.1		27.2	32.8	
March 4					82					29.4	33.0	

* 150 cc. washed dog's red blood cells laked with equal quantity of distilled water given through stomach tube at the beginning of the third hour.

TABLE F

Cooked pig blood feeding and bile pigment secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 29	26	29	29		84	7.6	10.4	9.1		27.1	32.3	
March 30	35	34*	34	28	96	10.2	9.2*	10.0	8.2	27.4	32.5	200 cc. cooked blood
March 31	25	36	31		92	9.0	12.0	9.1		30.1	32.5	
April 1					48					25.8	32.8	
April 3	22	21	31		74	10.4	11.5	10.4		32.3	32.0	Hemoglobin 116 per cent
Dog 16–41. Bile fistula and splenectomy												
March 24	35	55	25		115	11.0	14.1	7.3		32.4	37.3	
March 25					94					33.2	36.8	
March 27	36	30	28		94	14.4	13.6	10.6		38.6	37.0	Hemoglobin 93 per cent
March 28	19	18*	30	26	74	14.4	14.4*	12.8	11.6	38.8	36.8	350 cc. cooked blood
March 29	29	35	38		102	9.2	11.0	10.3		30.5	37.3	

* Cooked pig's blood given through stomach tube at the beginning of the third hour.

TABLE G

Digested ox red cell feeding and bile pigment secretion

Dog 16–10. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 29	22	31	23		76	13.8	13.6	14.6		42.0	32.0	
March 30	19	21*	27	29	77	14.6	14.4*	14.6	12.4	41.4	32.5	
March 31	19	23	25		67	11.0	12.9	12.4		36.3	32.3	
April 1					48					37.8	32.3	
April 3	19	20	28		67	14.1	11.6	15.8		41.5	30.8	Hemoglobin 102 per cent

* 100 cc. digested ox red blood cells given through stomach tube at the beginning of the third hour. Blood cells digested at 38°C. with pancreatin.

TABLE H

Digested ox blood feeding and bile pigment secretion

Dog 16-41. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 18					46					32.2	38.0	
March 20	38	32	35		105	12.0	10.8	10.2		33.0	37.0	Hemoglobin 94 per cent
March 21	20	37*	37	40	114	10.8	11.3*	7.3	7.2	25.8	37.3	100 cc. digested blood
March 22	46	33	40		119	10.4	9.7	9.0		29.1	38.0	
March 29	29	35	38		102	9.2	11.0	10.3		30.5	37.3	
March 30	28	25*	40	37	102	10.6	9.0*	11.6	9.7	30.3	37.0	150 cc. digested blood
March 31	21	24	28		73	8.4	9.2	9.4		27.0	37.3	
April 1					46					31.6	37.3	
April 3	41	22	28		91	12.2	8.9	8.8		29.9	36.3	Hemoglobin 99 per cent

* Digested whole ox blood given by stomach tube at the beginning of the third hour. The blood was digested for weeks with pancreatin at 38°C.

EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments had been under observation following a uniform routine for months and some for over a year. They were all in good condition with constant body weight, normal blood and uniform bile pigment secretion. Unless otherwise noted, these dogs showed only a trace of bile pigment in the urine, no stercobilin in the feces and no urobilin in the urine. Under such conditions a small variation in bile pigment output is worth careful consideration, provided the observation is constant in repeated experiments.

It may seem that repeated experiments with blood of different animals are not called for. It should be noted in other papers to follow that bile from different animals may have a constant effect on total bile secretion in the dog when given by mouth, but that bile of one animal may stimulate and bile of another animal may inhibit the bile pigment secretion. For this reason we report the observation on blood feeding in the dog, using blood from different animals.

DISCUSSION

The tables given above speak for themselves, and the results are quite uniform. Many more experiments have been done with this same material, and the results are identical. These other tables are not given for lack of space, but we feel that a sufficient number of experiments have been performed to make the point quite certain, and only a few type experiments are here recorded.

Blood feeding has no influence on the secretion of bile pigment in the normal dog. Whole blood of the sheep, pig or ox freshly defibrinated given to the dog by stomach tube does not influence the output of bile pigments nor the volume of bile. Freshly laked dog red cells given by stomach tube likewise have no effect on bile secretion. Cooked pig blood and digested ox blood have not the slightest influence on the output of bile pigments. There is no immediate effect nor any delayed influence on the bile secretion.

This diet is very rich in the pyrrol nucleus, perhaps contains it in as concentrated form as it occurs in normal body tissues or fluids. It seems safe to assume that during the period of observation a certain amount of this material was digested, and its constituent parts taken in through the intestinal mucosa. We propose to show that other substances in the diet besides carbohydrates will cause a rise in bile pigment output, but hemoglobin is not one of this group. It may be possible in the near future to indicate from what "building stones" the body constructs its various pigments which are so closely related. Perhaps the story may yet be written of how certain substances may be used when needed in pigment building or put aside in reserve as certain by-products or eliminated from the body in the form of one or another pigment.

CONCLUSION

The flow of bile and the secretion of bile pigments in the dog are not influenced by the ingestion of fresh blood of the dog, sheep, pig or ox.

Freshly laked red cells by mouth likewise have no effect.

Cooked pig blood or digested ox blood given by stomach have no influence on the secretion of bile pigments.

These negative findings are of some importance in view of the fact that carbohydrates by mouth cause an increased secretion of bile pigments in dogs.

Splenectomy causes many interesting reactions in bile fistula dogs, but it does not influence the output of bile pigments under normal conditions. Splenectomy has no effect upon these blood feeding experiments.

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ERRATUM

Pages 263 and 264 were imperfectly printed in some copies of the January issue (Vol. XLII, part 2, 1916–1917). As these copies cannot be traced, the pages in question are here reproduced so that all sets of this volume may be made perfect before binding.

BILE PIGMENT METABOLISM

IV. INFLUENCE OF FRESH BILE FEEDING UPON WHOLE BILE AND BILE PIGMENT SECRETION

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This paper presents the experimental data resulting from feeding fresh bile from various animals to dogs with bile fistulae. Bile feeding causes a very definite increase in bile flow from the fistula, as is well known, but also, with few exceptions, it causes a definite *diminution* of the outflow of bile pigments in spite of active cholagogue action, which might be expected to sweep an excess of bile pigments out of the liver. This sweeping out of an excess of bile pigments during cholagogue action is exactly what we might expect, provided the liver had simply an eliminative function in disposing of worn out corpuscles and hemoglobin by way of the bile. In the following paper the various constituents of bile are tested on bile fistula dogs, and the striking effects are analysed.

That bile feeding has a definite and striking effect on the bile pigment secretion is established, and is of considerable importance in understanding this obscure story of pigment metabolism. Can we assume that bile feeding causes a decrease in the continuous disintegration of red cells which we are told is going on with such rapidity in the body, and is solely responsible for the outpouring of bile pigment? There may be a reasonable doubt on this point. It is to be recalled that most of the evidence concerning the short life cycle of the red cell is based on end product determinations. If there is a suspicion that other substances than hemoglobin may be formed into these same end products or that some of the used up hemoglobin may not be formed into this same end product, we may also question the life cycle of the red cell as determined in this manner. At least we may say that there is a possibility of error, and assume that the life of a red cell *may* be normally more than ten to fourteen days. This important point will be taken up in later communications.

It is proper at this point to take up the question of "bile circulation" as applied to the bile pigments. Wertheimer (1) injected sheep's bile into dogs' portal veins, and showed that cholohematin appeared promptly in the dog's bile, and could be recognized by its characteristic spectrum. Curiously enough many writers quote this work as evidence that the bile is absorbed from the *intestine*, and that there is a circulation of bile pigments as well as bile salts. Stadelmann (2) leaves the question open, and says that very small amounts of bile pigment *may* be absorbed from the intestine. All our experiments give not the slightest evidence of any bile pigment absorption from the intestine, and the evidence seems sufficient to rule out any such possibility in dogs. We feel safe in claiming that there is no "bile circulation" of the bile pigments as such.

Another type of pigment circulation has been suggested by Addis (3), and has a strong appeal because of its very simplicity and directness. He suggests that the following outline may cover much of the life history of hemoglobin pigment. Hemoglobin is constantly liberated in the body from the disintegration of worn out red cells. This hemoglobin is changed in the liver to bile pigments, and excreted into the intestine. The bile pigment is reduced to urobilinogen in the intestine, a part escaping in the feces, and an important part being absorbed into the blood, polymerized into urobilin-complex, and taken up by the liver. This portion is stored in the liver or synthesized into hemoglobin, or, if the liver is abnormal, may escape into the blood and appear in the urine as urobilinogen. This hypothesis in a word is "bile circulation" again presented in a more elaborate form—a circulation of a pyrrol complex.

A part of this cycle may be admitted as true, that the pigment radicle of hemoglobin may appear in the bile as bile pigment and later as stercobilin or urobilinogen in the intestine. Is there any evidence that urobilinogen is absorbed from the intestine into the blood? Not one grain of direct evidence, as urobilinogen has never been demonstrated in portal blood. It is claimed that the stercobilin of the feces is insufficient to account for all the bile poured into the duodenum, therefore, some of it must be absorbed. There are several unknowns in this equation. It is claimed that urobilin is formed only in the intestine, and must be absorbed from it, because persons with common duct obstruction do not show urobilinogen nor stercobilin. It is also known that many other disorders result from exclusion of the bile from the intestine, and one could assume that such disturbances might react on other organs. It is possible to assume that urobilinogen may be produced at times in the liver (like other pigments), and that this production may depend in part on liver function (liver disease). There is not a bit of evidence produced so far which will not fit this hypothesis as well or better than the usual theory that urobilin is formed only in the intestine.

There is no direct evidence at hand to contradict us if we assume that urobilin under certain conditions may be produced outside of the intestine, perhaps in the liver or even kidney. With the evidence at hand to indicate that the production of one common body pigment (bilirubin) is possible outside of the liver (4), we must be guarded in our statements that some other common pigment (urobilin) may not be formed under a variety of conditions, particularly abnormal conditions.

Addis states that the importance of hemoglobin presupposes conservation by the body of this unusual pyrrol group. This may be granted as desirable for the sake of argument, but direct evidence of this must be brought forward before the hypothesis can be accepted. There is no direct evidence that urobilinogen is absorbed from the intestine, and the indirect evidence is capable of other interpretation.

There is strong indirect evidence against this intestinal absorption and "circulation" of the pyrrol group. Large doses of bile by mouth in bile fistula dogs cause *decrease* in bile pigment output. But much more important is the fact that dogs under observation for months and years with bile excluded from the intestine fail to develop an anemia. Such dogs over periods of months have been kept so that the feces were free from stercobilin, yet no anemia developed. This shows that the dog is in no way dependent for its supply of hemoglobin upon pigments absorbed from the intestine. These experiments do not exclude the possibility of any pigments being absorbed from the intestine, but they do show that if such absorption does take place it is of trivial importance in the pigment metabolism. The burden of proof must now rest with those who claim that there is some pigment absorption from the intestine in view of the fact that normal pigment metabolism goes on when all such absorption has been prevented over long intervals of time.

EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments make up the same group used in the preceding paper. In all cases bile was absent from the urine or present at the most in traces. Stercobilin as a rule was absent unless a note is made to the contrary. The routine treatment and diet of the dogs have been described previously (5), also the methods of collection and analysis. The dogs were all in good condition, and were in no way upset by these various experimental procedures, so it is not necessary to give protocols except in the form of tables.

Tables 1 and 2 show a pretty constant and sometimes an extreme cholagogue action following the introduction of dog bile by the stomach tube. There is a striking fall in bile pigment secretion appearing after two or four hours or sometimes as promptly as the cholagogue action during the first two hours. This fall is all the more noticeable as it appears during a high bile output. The bile pigment secretion is normal by the next day, but sometimes the cholagogue action seems to persist into the second day. Splenectomy does not influence this reaction.

Tables 3, 4 and 5 show the variation which may be noted after the feeding of dog bile. We have many more experiments, but the ones submitted here are good examples of two main groups. One group (tables 1 and 2) show more or less cholagogue action and a definite depression of bile pigment output. The other group (tables 3, 4 and 5) shows the same cholagogue action but little depression of the bile pigment secretion. There are border line experiments between these two groups. The reason for this comes out in the next paper of this series. It will be seen in the above tables that even if the total pigment output is equal to the average of previous days, there is a tendency toward a falling curve of secretion (table 3) following the ingestion of bile.

Table 6 gives the results of two experiments of a group of four. The results in each experiment were identical. The bile feeding by stomach tube was given in the early morning, and the bile collection done nine hours later. It is clear that there was no influence upon the output of bile pigments—no delayed reaction. One dog shows a cholagogue action and the other is negative. These experiments are necessary to meet the possible objection that the inhibition of bile pigment secretion might be only temporary to be followed by a proportional increase within a few hours.

TABLE 1

Dog bile feeding and bile secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
March 3	35	48	35		118	12.8	10.2	11.8		34.8	30.8	Stools contain stercobilin
March 4					96					30.6	31.0	
March 6	30	30	35		95	8.8	10.0	9.4		28.2	30.5	
March 7	20	67*	33	26	126	9.4	9.0*	2.9	0.8	12.7	30.0	
March 8	25	28	28		81	8.5	8.8	10.0		27.3	30.5	Hemoglobin 112 per cent
April 12	15	23	27		65	8.8	9.4	12.8		31.0	31.0	
April 13	17	25†	32	34	91	10.6	10.2†	3.6	2.3	16.1	30.5	
April 14	18	29	29		76	11.3	13.1	12.4		36.8	30.8	
April 15					42					32.0	31.0	
April 17	28	24	24		76	9.4	9.2	11.5		30.1	31.0	Hemoglobin 118 per cent

* 250 cc. dog's bile containing 104 mgm. bile pigments given through stomach tube at beginning of third hour.

† 200 cc. fresh dog bile containing 68 mgm. bile pigments given through stomach tube at beginning of the third hour.

TABLE 2

Dog bile feeding and bile secretion

Dog 16–10. Bile fistula and splenectomy

DATE	BILE										Weight	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 18					58					35.2	32.0	Hemoglobin 99 per cent.
March 20	20	23	24		67	12.2	11.4	12.4		36.0	31.8	Stools contain no stercobilin
March 21	22	55*	53	27	135	11.4	10.0*	6.0	6.7	22.7	31.8	
March 22	26	27	21		74	12.6	11.5	13.0		37.1	32.0	
April 12	18	24	28		70	10.2	11.9	11.3		33.4	31.0	Stools contain no stercobilin
April 13	16	31†	25	28	84	12.2	9.8†	2.8	1.9	14.5	31.0	
April 14	33	26	27		86	10.4	9.4	9.1		28.9	30.5	
April 15					54					33.6	30.8	Hemoglobin 104 per cent

* 350 cc. fresh dog bile containing 130 mgm. bile pigment given through stomach tube at the beginning of the third hour.

† 200 cc. fresh dog bile containing 68 mgm. bile pigments given through stomach tube at the beginning of the third hour.

TABLE 3

Dog bile feeding and bile secretion

Dog 16–41. Bile fistula and splenectomy

DATE	BILE										Weight	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
March 15	48	36	27		111	10.5	11.2	11.5		33.2	37.8	
March 16	44	84*	70	53	207	16.3	13.0*	17.2	4.8	35.0	38.3	
March 17	46	38	46		130	13.1	12.1	12.7		37.9	38.5	
March 18					46					32.2	38.0	
March 20	38	32	35		105	12.0	10.8	10.2		33.0	37.0	Hemoglobin 94 per cent (Sahli)
March 3	30	36	33		99	10.0	11.2	9.7		30.9	37.3	
March 4					90					30.6	37.5	
March 6	37	25	31		93	10.5	9.0	11.8		31.3	36.8	Hemoglobin 79 per cent
March 7	28	37†	37	30	104	11.3	11.3†	7.3	4.6	23.2	37.0	
March 8	18	30	27		75	9.4	12.1	9.7		31.2	37.3	

* 450 cc. fresh dog bile containing 140 mgm. bile pigment given through stomach tube at beginning of the third hour.

† 150 cc. fresh dog bile containing 70 mgm. bile pigments given through stomach tube at the beginning of the third hour.

TABLE 4

Dog bile feeding and bile secretion

Dog 16-6. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Bread, bone and milk diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.		
1916											*lbs.*	
Nov. 1	16	18	20		54	10.8	13.8	11.9		36.5	31.5	
Nov. 3	17	18	20		55	13.4	11.3	15.0		39.7	31.5	Stools contain no stercobilin
Nov. 4	22	20	22		64	12.9	13.2	13.1		39.2	31.0	
Nov. 5	18	32*	21	17	70	12.6	10.1*	10.9	10.7	31.7	30.8	
Nov. 6					66					34.1	30.8	

Dog 16-27. Bile fistula and splenectomy

DATE	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	WEIGHT	REMARKS
Feb. 26					32					22.2	26.0	Mixed diet
Feb. 28	10	20	23		53	7.5	10.4	8.2		26.1	25.0	Hemoglobin 81 per cent
Feb. 29	19	47†	34	22	103	10.4	10.6†	6.7	7.6	24.9	25.8	
March 1	14	19	22		55	8.8	8.9	9.4		27.1	25.5	

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* 45 cc. fresh dog's bile introduced into empty stomach through stomach tube at beginning of the third hour.

† 200 cc. fresh dog bile containing 140 mgm. bile pigment given through stomach tube at beginning of the third hour.

TABLE 5

Dog bile feeding and bile secretion

Dog 16–104. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
Feb. 25	20	15	17		52	12.7	8.1	11.4		32.2	33.0	
Feb. 26					46					28.0	32.5	
Feb. 28	14	17	18		49	8.5	13.0	13.4		34.9	32.8	Hemoglobin 96 per cent
Feb. 29	16	44*	28	23	95	10.0	13.8*	7.0	8.8	29.6	32.5	
March 1	25	19	19		63	10.2	8.9	8.9		28.0	33.0	
Dog 16–10. Bile fistula and splenectomy												
Feb. 21	13	15	17		45	10.6	11.0	11.8		33.4	30.3	Hemoglobin 94 per cent
Feb. 23	19	18	27		64	10.4	8.1	11.6		30.1	30.5	
Feb. 24	12	40†	40	34	114	9.6	11.3†	10.5	6.2	28.0	30.3	
Feb. 25	22	27	21		70	9.4	12.8	11.5		33.7	30.8	
Feb. 26					42					30.4	31.0	Stools contain no stercobilin

* 100 cc. fresh dog bile containing 70 mgm. bile pigment given through stomach tube at beginning of third hour.

† 360 cc. fresh dog bile containing 198 mgm. bile pigments given through stomach tube at beginning of the third hour.

TABLE 6

Dog bile feeding nine hours before collection

Dog 15–22. Simple bile fistula

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1916									*lbs.*	
April 5	22	29	21	72	10.9	9.2	9.4	29.5	32.5	
April 6*	19	27	22	68	8.9	13.4	8.9	31.2	32.0	
April 7	25	21	21	67	12.4	9.4	11.3	33.1	32.3	Stools contain stercobilin
April 8				54				31.6	32.0	
April 10	18	24	22	64	8.1	11.5	9.9	29.5	31.5	Hemoglobin 116 per cent
Dog 16–27. Bile fistula and splenectomy										
April 5	18	19	27	64	6.8	8.6	12.1	27.5	26.5	
April 6*	32	34	35	101	11.6	6.2	8.6	26.4	26.5	
April 7	17	23	29	69	7.7	8.2	8.6	24.5	26.3	
April 8				50				24.2	26.3	
April 10	18	22	27	67	8.4	8.9	8.5	25.8	25.5	Hemoglobin 94 per cent

* 12.30 a.m. 300 cc. fresh dog bile containing 135 mgm. bile pigments given through stomach tube. Dogs set up at 9.30 a.m. Bile output nine hours after feeding dog bile.

TABLE 7

Pig bile feeding and bile secretion

Dog 16–27. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
											lbs.	
1916												
March 22	18	17	24		59	6.0	5.7	6.4		18.1	26.3	
March 23	29	24*	13	13	50	8.6	3.8*	1.2	0.9	5.9	26.8	
March 24	35	40	23		98	6.3	7.1	5.2		18.6	26.5	
March 25					40					25.4	25.8	Stools contain no stercobilin
April 12	21	27	26		74	6.2	7.9	6.4		20.5	26.3	
April 13	8	27†	27	27	81	6.4	9.7†	3.0	1.8	14.5	26.0	
April 14	27	27	27		81	7.9	6.0	6.7		20.6	26.0	
April 15					66					20.8	26.3	Hemoglobin 95 per cent

* 150 cc. fresh pig bile containing 65 mgm. bile pigment fed through stomach tube at the beginning of the third hour.

† 200 cc. fresh pig bile containing 68 mgm. bile pigments fed through stomach tube at the beginning of the third hour.

TABLE 8

Pig bile feeding and bile secretion

Dog 16–41. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 22	46	33	40		119	10.4	9.7	9.0		29.1	38.0	
March 23	47	44*	47	50	141	12.4	8.0*	5.4	3.4	16.8	38.0	
March 24	35	55	25		115	11.0	14.1	7.3		32.4	37.3	
March 25					94					33.2	36.8	
March 27	36	30	28		94	14.4	13.6	10.6		38.6	37.0	Hemoglobin 93 per cent
Dog 16–60. Simple bile fistula												
April 12	24	21	23		68	6.4	4.7	6.2		17.3	38.5	
April 13	28	30†	40	34	104	7.6	4.0†	2.8	1.9	8.7	38.8	
April 14	16	28	23		67	5.4	10.6	3.1		19.1	37.5	
April 15					36					15.4	38.5	Hemoglobin 98 per cent
April 17	20	19	21		60	9.0	7.2	5.2		21.4	38.3	

* 150 cc. fresh pig bile containing 65 mgm. bile pigment fed through stomach tube at beginning of the third hour.

† 200 cc. fresh pig bile containing 68 mgm. bile pigment fed through stomach tube at the beginning of the third hour.

TABLE 9

Sheep bile feeding and bile secretion

Dog 16–10. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
March 22	26	27	21		74	12.6	11.5	13.0		37.1	32.0	
March 23	26	57*	54	54	165	13.4	11.3*	5.4	3.6	20.3	32.3	
March 24	29	36	21		86	12.4	15.8	10.9		39.1	32.0	Stools contain stercobilin
March 25					56					37.6	31.5	
March 27	17	17	28		62	13.0	8.8	15.2		37.0	31.0	Hemoblogin 97 per cent.
Dog 15–22. Simple bile fistula												
March 22	35	35	26		96	10.4	9.2	9.7		29.3	31.5	
March 23	22	58†	40	14	112	10.4	10.2†	3.6	7.6	21.4	31.5	
March 24	35	35	21		91	12.0	11.0	6.2		29.2	31.5	Stools contain stercobilin
March 25					94					28.8	32.0	Hemoglobin 119 per cent.
March 27	22	26	25		73	10.4	11.6	8.5		30.5	31.5	

* 150 cc. fresh sheep bile containing 29 mgm. bile pigments given through stomach tube at beginning of the third hour.

† 150 cc. fresh sheep bile containing 29 mgm. bile pigments given through stomach tube at beginning of the third hour.

TABLE 10

Ox bile feeding and bile secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
March 10	24	25	25		74	9.8	9.0	8.5		27.3	31.3	
March 11					64					26.4	31.5	
March 13	20	25	19		64	9.9	10.8	7.6		28.3	31.3	Hemoglobin 114 per cent
March 14	34	60*	63	41	164	12.2	13.6*	5.6	7.3	26.5	31.5	
March 15	35	27	30		92	13.3	8.2	10.7		32.2	31.5	

Dog 16–41. Bile fistula and splenectomy

DATE	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	WEIGHT	REMARKS
March 8	18	30	27		75	9.4	12.1	9.7		31.2	37.3	
March 9	35	75†	76	77	228	14.1	13.5†	7.2	5.1	25.8	37.3	
March 10	13	15	30		58	10.2	7.7	12.8		30.7	37.0	
March 11					58					32.8	37.3	
March 13	17	27	31		75	9.2	10.3	9.8		29.3	37.0	Hemoglobin 81 per cent

* 200 cc. fresh ox bile containing 51 mgm. bile pigments given through stomach tube at the beginning of the third hour.

† 250 cc. fresh ox bile containing 73 mgm. bile pigment given through stomach tube at end of the third hour.

Tables 7 and 8 show a pretty uniform reaction as regards the decrease in bile pigment secretion. The decrease is often over 50 per cent and the reaction very prompt, appearing usually in the first two hours. The fall during the two hour periods is remarkable, and indicates some very strong inhibiting substance which checks the outflow of bile pigments, while the volume of bile is unchanged or increased. Evidently pig's bile is different in some way from dog's bile as regards its physiological action on dogs.

Tables 9 and 10 show that sheep and ox bile have a tendency to depress the curve of bile pigment secretion but not comparable to the strong effect produced by feeding pig bile. Ox bile has the strongest cholagogue effect of any bile tested.

DISCUSSION

The experiments tabulated above are pretty uniform, and in regard to certain points are capable of only a single interpretation. These experiments practically rule out any "bile circulation," meaning the absorption of bile pigment as such from the intestine. The experiments give no support to the theory of Addis that the pyrrol nucleus is absorbed from the intestine as urobilinogen, and carried to the liver as an important construction element for hemoglobin—a "bile circulation" of a pyrrol complex. In fact, these experiments give strong indirect evidence against this theory of Addis. The evidence is strong against the absorption of urobilinogen from the intestine, and all the known facts will fit a different theory, namely, that urobilin like bile pigments may at times be formed in one organ or another from some related pigment substance. Direct proof that urobilinogen can be absorbed from the intestine in considerable amounts is needed to render Addis' theory a useful hypothesis.

These experiments show the usual cholagogue action of fresh bile by mouth, but it is to be noted that even this well-known reaction is not absolutely constant. Ox bile in these experiments is the most active cholagogue, but all fresh bile will show considerable variation.

The inhibition of bile pigment output in spite of the cholagogue action is as constant as the direct cholagogue effect and equally pronounced. Fresh bile by mouth often doubles the bile flow and as frequently *decreases* by 50 per cent the outflow of bile pigments. Pig bile is most active in producing this inhibition of bile pigment secretion. The fall in output of pigments usually appears promptly, and the effect disappears within nine hours. We are not prepared to explain the full significance of this constant reaction, but, to say the least, it strengthens our belief that bile pigments are formed in some other manner than through disintegration of red cells and hemoglobin.

As we find more substances (organic or inorganic) which influence promptly the secretion of bile pigments, may we not suspect that these pigments are manufactured in considerable degree by some organ which reacts promptly to various stimuli? It requires the widest stretch of the imagination to picture these various simple substances as influencing the red cell katabolism—one group causing prompt and extensive red cell destruction, and the other group equally prompt inhibition. Is it not very probable that these substances react on an organ busy in the production of pigments, and cause it to produce more or less pigment of a certain type?

SUMMARY

Feeding fresh bile to bile fistula dogs causes an almost constant cholagogue action. Bile of the dog, sheep and pig all have this effect, and ox bile seems to be the most active cholagogue.

Fresh bile feeding as a rule causes a *fall in the output* of *bile pigments* in spite of the cholagogue action. Pig bile as a rule is most active in this respect, and may depress the bile pigment curve to less than half normal. Ox and sheep bile may have the same effect. Dog bile often causes only a slight fall in bile pigment output, but may depress the curve to 50 per cent of normal.

These experiments exclude the possibility of bile pigment absorption from the intestine. There is no evidence to support the theory of "bile pigment circulation." These experiments give no support to Addis' theory of pigment conservation, urobilinogen absorption from the intestine and synthesis toward hemoglobin. It is suggested that urobilinogen may be formed in other body tissues rather than absorbed from the intestine.

Splenectomy does not influence any of the above reactions in bile fistula dogs.

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BILE PIGMENT METABOLISM

V. THE INFLUENCE OF BILE CONSTITUENTS ON BILE PIGMENT SECRETION, TAUROCHOLIC, GLYCOCHOLIC AND CHOLIC ACIDS AND BILE FAT

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The preceding paper makes it clear that bile feeding may give somewhat variable results under pretty uniform conditions. We were particularly interested in the fact that pig bile almost always caused a remarkable decrease in bile pigment output in dogs with biliary fistulae, but dog bile had a less pronounced inhibiting effect or even no effect on bile pigment secretion. It is well known that bile of herbivorous animals differs in its chemical make-up from the bile of carnivorous animals. It was suspected that some of these variations in bile secretion following bile feeding might be explained by differences in chemical constitution of the ingested bile.

Any chemical substances capable of influencing the bile pigment secretion seemed worthy of investigation. Some of the more conspicuous elements of whole bile were isolated and tested in the experiments outlined below. That these substances have distinctive physiological activities is obvious, and that some of these have a definite action upon bile pigment secretion is equally clear. It is admitted that these experiments touch on only a few of the well known elements of normal bile, and many other constituents should be investigated. We hope to report further on these points in the future.

The experiments given below confirm those of the preceding paper in showing how profoundly and promptly the bile pigment output can be influenced by ingested substances. Further than that these experiments show that taurocholic acid has more of a cholagogue action than glycocholic acid but much less, if any, action on bile pigment secretion. Glycocholic acid is a less active cholagogue, but causes pronounced inhibition of bile pigment output. Bile fat (simple ether extract) is also capable of depressing the curve of bile pigment secretion, while influencing in no degree the total output of bile.

It is probable that cholesterol is an inert substance as regards any control of bile pigment secretion. Some experiments were performed in which cholesterol (1–2 grams) were added to the daily food but with negative results. These experiments are not conclusive, and the question may be left open at present with a strong probability that cholesterol is a negative factor in bile pigment secretion.

EXPERIMENTAL DATA

The same bile fistula dogs are used in the experiments as in the experiments described in the two previous papers. Unless note is made to the contrary, the same conditions obtain. The urine never contained more than a trace of bile pigments and no urobilin.

The taurocholic and taurocholeic acids were prepared from dog bile. The whole bile mixed with an excess of animal charcoal was evaporated to dryness at 70° to 80°C. The residue was extracted several times with absolute alcohol, and the alcoholic filtrate decomposed with hydrochloric acid and precipitated with ether. The bile acids were purified by redissolving in alcohol and reprecipitating with ether.

The glycocholic acid was prepared from fresh pig's bile, evaporating it to dryness in the presence of an excess of animal charcoal at 70° to 80°C., extracting the dried residue with absolute alcohol and precipitating the filtrate with dilute hydrochloric acid and water. The glycocholic acid was purified by redissolving in alcohol and precipitating with water.

The cholic acid used was prepared from glycocholic acid by hydrolysis with hydrochloric acid. The cholic acid fraction was rubbed up with distilled water, and then cold alcohol, and finally precipitated in relatively pure form from hot alcohol.

The bile fat was obtained from dog bile. The whole bile mixed with an excess of animal charcoal was evaporated to dryness, and the residue extracted with ether.

TABLE 21

Taurocholic acid feeding and bile secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
March 17	33	32	27		92	12.0	10.8	9.1		31.9	30.8	
March 18					52					32.8	31.5	
March 20	25	24	31		80	8.9	9.6	10.4		29.9	31.5	Hemoglobin, 116 per cent
March 21	19	53*	29	19	101	9.4	10.6*	9.2	9.9	29.7	31.5	5 grams taurocholic acid
March 22	35	35	26		96	10.4	9.2	9.7		29.3	31.8	
Dog 16–27. Bile fistula and splenectomy												
March 31	10	20	27		57	7.2	8.6	8.5		24.3	26.5	
April 1					44					18.8	26.5	
April 3	17	22	29		68	5.7	6.4	5.8		17.9	26.0	Hemoglobin, 92 per cent
April 4	22	44*	36	30	110	5.9	6.0*	4.8	5.2	16.0	26.5	3* grams taurocholic acid.
April 5	18	19	27		64	6.8	8.6	12.1		27.5	26.5	

* Taurocholic and taurocholeic acids dissolved in 400 cc. water given through stomach tube at the beginning of the third hour.

TABLE 22

Taurocholic acid feeding and bile secretion

Dog 16–10. Bile fistula and splenectomy

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 31	19	23	25		67	11.0	12.9	12.4		36.3	32.3	
April 1					48					37.8	32.3	
April 3	19	20	28		67	14.1	11.6	15.5		41.5	31.8	Hemoglobin, 102 per cent
April 4	20	43*	31	24	98	13.6	19.8*	11.1	11.0	41.9	31.5	
April 5	21	25	26		72	12.2	12.9	14.0		39.1	32.0	
Dog 16–41. Bile fistula and splenectomy												
March 31	21	24	28		73	8.4	9.2	9.4		27.0	37.3	
April 1					46					31.6	37.3	
April 3	41	22	28		91	12.2	8.9	8.8		29.9	36.3	Hemoglobin, 99 per cent
April 4	41	63*	51	47	161	12.8	13.6*	13.4	10.8	37.8	37.0	
April 5	29	32	42		103	9.2	10.4	11.8		31.4	37.0	

* 3 grams taurocholic and taurocholeic acids dissolved in 600 cc. water given through stomach tube at the beginning of the third hour.

TABLE 23

Glycocholic acid feeding and bile secretion

Dog 16–60. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		Mixed diet
1916											*lbs.*	
April 6	24	7	45		76	9.2	1.6	7.2		18.0	38.3	
April 7	22	16	29		67	5.4	4.4	7.2		17.0	38.8	
April 8					66					22.4	38.3	
April 10	20	14	29		63	4.6	3.8	7.2		15.6	37.3	Hemoglobin, 101 per cent
April 11	23	30*	27	35	92	5.7	2.7*	1.8	2.4	6.9	38.5	
April 12	24	21	23		68	6.4	4.7	6.2		17.3	38.5	
Dog 16–27. Bile fistula and splenectomy												
April 7	17	23	29		69	7.7	8.2	8.6		24.5	26.3	
April 8					50					24.2	26.3	
April 10	18	22	27		67	8.4	8.9	8.5		25.8	25.5	Hemoglobin, 94 per cent
April 11	16	21*	17	19	57	8.2	6.2*	2.3	1.3	9.8	26.3	
April 12	21	27	26		74	6.2	7.9	6.4		20.5	26.3	

* 10 grams glycocholic acid dissolved in 250 cc. 1 per cent sodium carbonate solution fed through stomach tube at the beginning of the third hour.

TABLE 24

Glycocholic acid feeding and bile secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
April 7	25	21	21		67	12.4	9.4	11.3		33.1	32.3	
April 8					54					31.6	32.0	Stercobilin present
April 10	18	24	22		64	8.1	11.5	9.9		29.5	31.5	Hemoglobin 116 per cent
April 11	26	34*	23	26	83	10.0	10.0*	4.1	3.0	17.1	31.5	
April 12	15	23	27		65	8.8	9 4	12.8		31.0	31.0	
Dog 16–41. Bile fistula and splenectomy												
April 8					68					30.6	37.3	
April 10	22	22	37		81	10.4	10.4	13.7		34.5	36.3	Hemoglobin 110 per cent
April 11	27	29*	53	37	119	9.8	11.0*	2.5	3.1	16.6	37.0	
April 12	21	28	27		76	6.4	8.2	8.5		23.1	37.3	

* 10 grams glycocholic acid dissolved in 250 cc. 1 per cent sodium carbonate solution fed through stomach tube at the beginning of the third hour.

TABLE 25

Cholic acid feeding and bile secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
April 14	18	29	29		76	11.3	13.1	12.4		36.8	30.8	
April 15					42					32.0	31.0	
April 17	28	24	24		76	9.4	9.2	11.5		30.1	31.0	Hemoglobin 118 per cent
April 18	15	22*	27	27	76	9.1	10.4*	9.4	8.9	28.7	31.0	
April 19	29	29	26		84	9.2	8.6	7.6		25.4	32.0	
Dog 16–41. Bile fistula and splenectomy												
April 14	23	24	28		75	10.9	11.0	10.0		31.9	36.5	
April 15					56					30.4	37.3	Hemoglobin 106 per cent
April 17	20	23	28		71	9.4	10.3	11.3		31.0	36.0	
April 18	19	29*	15	21	65	3.4	6.5*	3.0	4.6	14.1	37.0	
April 19	22	29	32		83	8.6	8.6	10.1		27.3	37.5	

* 2 grams cholic acid emulsified in 200 cc. of a 1 per cent sodium carbonate solution given by stomach tube at beginning of third hour.

TABLE 26

Bile fat feeding and bile secretion

Dog 15–22. Simple bile fistula

DATE	BILE										WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams						Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.		
1916											*lbs.*	
March 31	25	36	31		92	9.0	12.0	9.1		30.1	32.5	
April 1					48					25.8	32.8	
April 3	22	21	31		74	10.4	11.5	10.4		32.3	32.0	Hemoglobin 116 per cent
April 4	25	23*	23	22	68	8.5	5.2*	5.7	5.6	16.5	32.3	
April 5	22	29	21		72	10.9	9.2	9.4		29.5	32.5	
Dog 16–27. Bile fistula and splenectomy												
March 17	25	25	29		79	8.5	7.3	7.2		23.0	26.5	
March 18					52					22.4	26.8	
March 20	9	19	24		52	8.8	8.5	8.6		25.9	26.3	Hemoglobin 85 per cent
March 21	20	17*	17	20	54	6.4	4.6*	3.8	3.9	12.3	26.5	
March 22	18	17	24		59	6.0	5.7	6.4		18.1	26.3	

* 1.1 gram bile fat emulsified in 200 cc. water fed through stomach tube at the beginning of third hour.

DISCUSSION

The above tabulated experiments make several points clear. Glycocholic acid has a moderate cholagogue action, but usually causes a great drop in bile pigment output in a bile fistula dog. Taurocholic acid has a strong cholagogue action, but little, if any, inhibiting effect on the bile pigment secretion. Bile fat (ether extract of whole bile) has no influence on bile flow, but causes inhibition of bile pigment secretion. Cholic acid has little effect on bile flow, but may decrease somewhat the bile pigment output.

With these facts in mind it may be possible to explain some of the obscure points noted in the preceding paper dealing with whole bile feeding. Pig bile is known to be very rich in glycocholic acid and very poor in taurocholic acid, so it is not surprising that pig bile given to bile fistula dogs usually causes a marked decrease in output of bile pigments. Dog bile is known to contain salts of taurocholic and taurocholeic acids but no glycocholic acid (at the most a trace). We understand now why dog bile does not cause as marked a decrease in bile pigment secretion as does pig bile. The ether extract of dog bile given to dogs with biliary fistulae usually causes a decrease in pigment output. This substance or group of substances may explain the tendency for dog bile feeding to cause a fall in bile pigment secretion. There may be substances in whole bile which are capable of increasing the outflow of bile pigments and their isolation and identification if present are much to be desired.

It may be objected that glycocholic acid is foreign to the dog's metabolism, and causes an unusual effect because of this fact. Such objections can not be raised against the other substances used, and the observations have some physiological interest.

SUMMARY

Taurocholic acid given by mouth has a strong cholagogue action but little influence upon the secretion of bile pigments in bile fistula dogs.

Glycocholic acid by mouth has a moderate cholagogue action but a strong inhibiting effect upon bile pigment secretion.

Cholic acid has little if any cholagogue action, but may decrease somewhat the bile pigment secretion in the bile fistula dog.

Bile fat (ether extract) has no cholagogue action, but has a strong inhibiting effect upon the secretion of bile pigments.

The results obtained in bile fistula dogs by feeding whole bile may depend upon these factors (and others). The end result (inhibition or stimulation) is probably the true resultant of these many forces. It is possible to explain some of the divergent results met with after bile feeding by this study of some of the constituent parts of whole bile.

OBSERVATIONS ON THE EFFECTS OF PARTIAL AND COMPLETE OCCLUSION OF BLOOD VESSELS ON THE GENERAL BLOOD PRESSURE IN MAN

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The effect on the general blood pressure of occluding and opening some of the larger arteries and veins in animals has been the subject of some study. So far as we are aware, however, observations of this kind have never been made in man. The present investigation was undertaken with a view to gathering information with regard to the effects on the general arterial pressure and heart rate of such procedures in man.

DESCRIPTION OF APPARATUS AND METHOD OF PROCEDURE

The principal method consisted in applying different known pressures to the vessels of both thighs while following the arterial pressure in the brachial artery by the continuous oscillatory method (1). Both thighs, instead of only one, were used so as to obtain unmistakable reactions.

The apparatus consisted of an Erlanger sphygmomanometer; a foot plethysmograph with volume recorder; an air tank connected with a compressed air system, with a mercury manometer and with two blood pressure cuffs for the application of pressure to the thighs; and a time recorder.

The plethysmograph was made in the form of a boot large enough to include the foot and the leg up to about the middle of the calf with which an air-tight joint was made by means of a piece of band tubing. The volume changes were recorded by means of a calibrated volume recorder. The tank was charged with air at the pressure it was desired to throw into the cuffs on the legs. Then by simply turning a stopcock this pressure could be suddenly applied to or removed from the legs. Through the mercury manometer the moment of application of pressure, the instant at which it was released and the time during which it was applied as well as the pressure itself were recorded. The effect upon the arterial blood pressure, upon the volume of the leg, the pressure exerted on the thighs and the time in seconds were recorded simultaneously.

The effect on the arterial pressure of applying the compression to and releasing it from the legs was followed by means of continuous blood pressure records made with the Erlanger sphygmomanometer according to a principle first employed by Erlanger (1). The subject of the experiment lay on his back upon a table; the right leg and foot were placed in the plethysmograph; a large blood pressure cuff was fastened around each thigh, as high up as possible; and the cuff of the Erlanger sphygmomanometer was placed on the left arm to record the changes in the amplitude of the oscillations obtained from the brachial artery. The tambour space of the sphygmomanometer was completely closed while recording the oscillations. Records to the number of 192 were made from two subjects at compression pressures ranging between 10 and 140 mm. Hg. The blood pressures of these subjects, both of whom were normal young men, were (E) average systolic pressure, 108 mm. Hg., average diastolic, 64; and (J) average systolic pressure 126 mm. Hg., average diastolic, 62.

RESULTS

Effect of compression on oscillation amplitude. The effects noted varied with the level of the compressing pressure. Applying compression to the thigh at the lower pressures causes no change in the amplitude of the oscillations obtained from the brachial artery by the continuous method. At somewhat higher compression, an effect upon the oscillations first becomes apparent as an increase in amplitude. This increase in amplitude first occurs at a compression pressure of 70 mm. Hg. and at this pressure it is found in 12.5 per cent of the trials. It is observed in a larger proportion of the trials as the compressing pressure is raised until at 100 mm. Hg. it occurs in every one of the records. At 70 mm. the increase in amplitude is delayed, not following immediately upon the application of pressure, but appearing several seconds afterward (fig. 1). As the compression pressure is increased the delay in the appearance of the increased oscillations decreases until at 140 mm. all of the compressions cause an immediate and marked effect (fig. 2). These results are tabulated below (table 1).

At the lower compressing pressures the increase in amplitude is of short duration, usually disappearing in the course of a few pulse beats. At pressures above 110 mm., however, (fig. 2) it shows a greater tendency to persist and in a considerable number of the records the effect persisted without appreciable diminution until the pressure was released, the maximum duration being two minutes and twenty-two seconds.

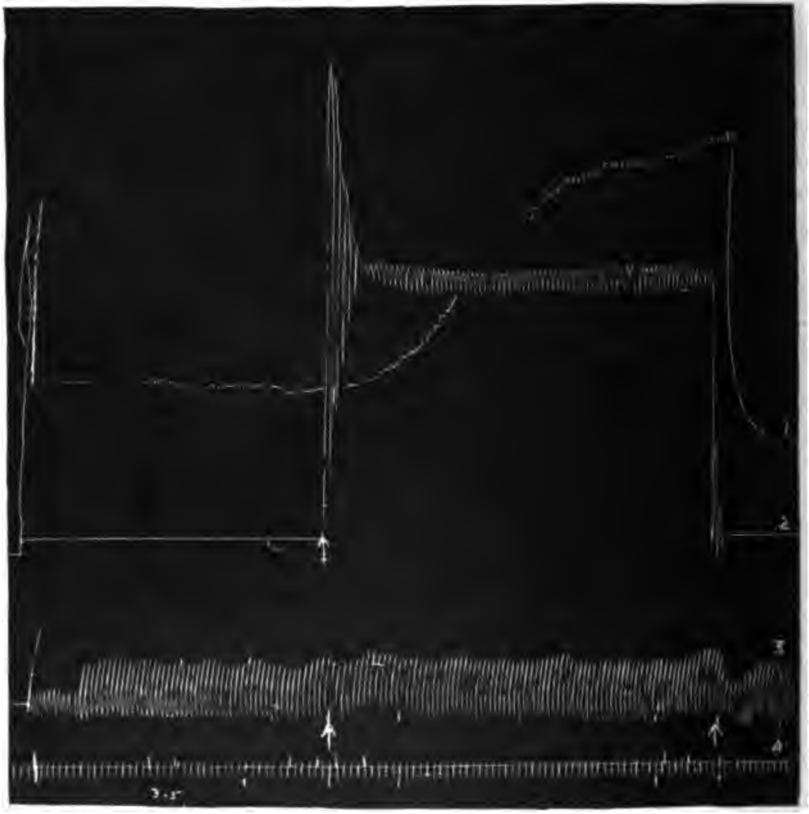

Fig. 1. Record to illustrate effect of a compression pressure of 70 mm. Hg. *1*, Volume of leg (right); *2*, compression pressure; *3*, sphygmomanometric record from left arm; *4*, time in seconds.

Effect of decompression on oscillation amplitude. The effect of releasing the pressure is more marked than the effect of applying it; it becomes evident at lower compression pressures and the response is

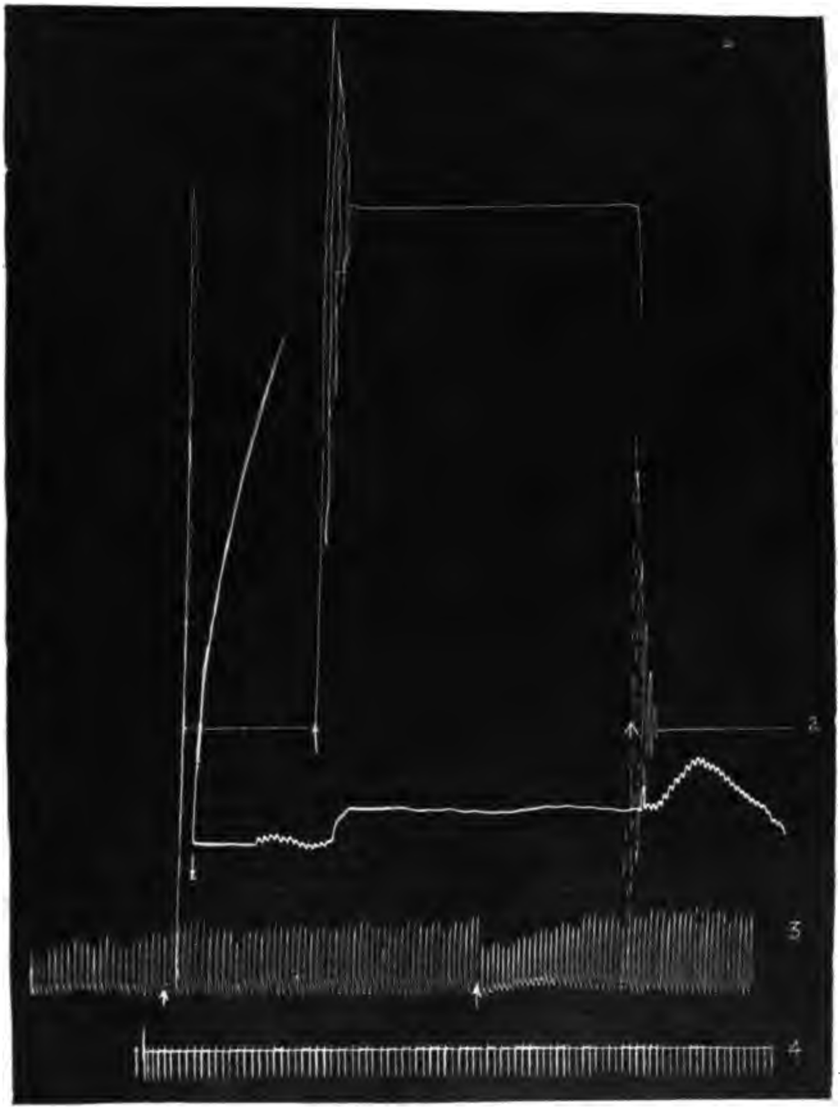

Fig. 2. Record illustrating effect of a high compression pressure. *1*, Volume of leg (right); *2*, compression pressure (140 mm. Hg.); *3*, sphygmomanometric record from left arm; *4*, time in seconds.

TABLE 1

PRESSURE IN MM. HG.	PER CENT OF TRIALS SHOWING		INCREASE IN VOLUME IN 15 SECONDS	TOTAL VOLUME INCREASE
	Increase in amplitude on compression	Decrease in amplitude on decompression		
			cc.	cc.
10	0	0	0	0
20	0	0	0	0
25	0	0	0	0
30	0	0	3.2	4
40	0	Occasional, slight	5.75	10.3
50	0	Occasional, slight	7.6	16.0
60	0	Definite in 36 per cent, no delay.	8.5	18.5
70	Definite in 12.5 per cent, delayed.	Definite in 62.5 per cent no delay.	9.7	24.0
75	Definite in 33.3 per cent, not delayed.	Definite in 66.6 per cent		
80	Definite in 28.5 per cent Some delayed	Definite in 85.7 per cent	10.25	26.6+
90	Definite in 57 per cent	Definite in 85.7 per cent	9.6	27.4+
100	Definite in 75 per cent Some abrupt	Definite in 100 per cent	9.7	29.0+
110	Definite in 57 per cent	Definite in 100 per cent	11.0	28.3+
120	Definite in 100 per cent, abrupt	Definite in 100 per cent	9.0	23.9
130	Definite in 100 per cent, abrupt	Definite in 100 per cent	3.8	5.5
140	Definite in 100 per cent, abrupt	Definite in 100 per cent	0	0

The data given in this table are based upon observations made on the subject (E). The observations made on (J) bear them out in every essential.

It must be remembered that the figures here given for total volume increase are only approximations and are much too low. More exact values might have been obtained with a different style of volume recorder.

The sign plus (+) where it appears in the table indicates that in a considerable number of cases the excursions of the recording lever were so wide as to cause it to leave the record. The values given are therefore very much too low.

The systolic pressure in the thigh was somewhat higher than that determined in the arm, averaging 114 mm. Hg.

immediate, despite the fact that the pressure is applied and released at approximately the same speed. The effect consists in a marked decrease in amplitude of the oscillations, and very often, more particularly at the higher pressures, a drop in the level of the base line of the record (figs. 1 and 2). This effect was noted with a pressure as low as 40 mm. Hg. and increased in frequency of occurrence with increasing pressure up to 100 mm. At and above this pressure it occurred in every case. The oscillations attain their lowest amplitude usually within one or two pulse beats and then return to their original size in the course of from 6 to 25 beats, usually about 12 beats. The effects of releasing the pressure are shown in table 1.

Effect of the duration of compression. For the purpose of determining the value of the time factor all the records made with compression pressures above 50 mm. Hg. were divided into groups, each group containing records having nearly the same compression pressure. Pressures below 55 mm. were not used because the effects at those pressures were very slight and thus difficult to measure, or absent altogether. There were nine groups in all. Group I contained records made with compressions of 55 to 63 mm. Hg.; group II, 68 to 75 mm. Hg.; group III, 75 to 80 mm. Hg.; group IV, 90 mm. Hg.; group V, 100 mm. Hg.; etc. up to group IX, 140 mm. Hg. An examination of all the records made with the object of determining the effect of the time during which compression was applied shows that the effect of releasing the pressure on the thighs varies directly with the length of time during which the pressure was applied in all the groups except I (55 to 63) and VIII (130). In group VIII, the time periods were not varied enough to form the basis of a fair comparison but as it appears in groups VII and IX it may safely be assumed that the time factor is present. Thus only group I is left in which no relation can be observed between the

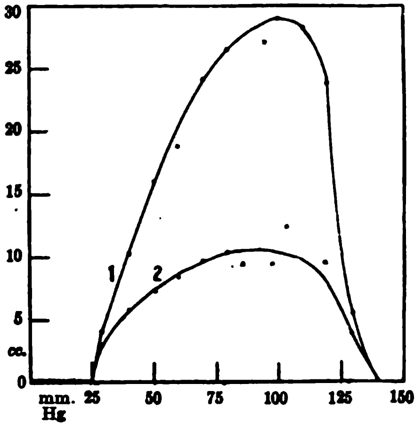

Fig. 9. Curves showing total volume increase (1) and increase in the first 15 seconds (2) at different compressing pressures. (Total volume increase for pressures of 80 to 100 mm. Hg. is too low. See footnote to table 1.)

length of time during which pressure was applied and the intensity of the effect of releasing it.

Significance of the changes in amplitude. That these changes in amplitude of the oscillations obtained from the brachial artery were indicative of changes in arterial blood pressure was definitely proven by a number of observations.

In the first place, to dispose of the possibility that the effects described might be due to any such cause as alterations in the position of the subject with resulting changes in the contact of the cuffs with extraneous objects, a series of experiments was performed in which the cuffs were so placed, and the body supported in such a manner that the blood pressure cuffs came into contact with nothing but the body. The records obtained in these experiments were in every respect similar to those obtained before.

Proof that compression and decompression cause changes in blood pressure was obtained by the use of the auscultatory method of determining the blood pressure. The pressure in the brachial artery was estimated by this method before and after compression, and again before and immediately after decompression. The compressions caused a rise in blood pressure of 4 to 8 mm. Hg., depending to some extent upon the pressure used. To detect the fall in blood pressure occurring as the result of decompression of the femoral vessels, the pressure in the brachial artery was determined about one minute after compression. Then, with the pressure in the sphygmomanometer about 2 mm. below systolic pressure, a pressure which just allowed the sounds to come through, the pressure on the thighs was suddenly released while listening to the arterial sounds in the brachial artery. The sounds disappeared. The pressure in the sphygmomanometer was then immediately allowed to fall, until the sounds returned. This occurred at a level which was from 4 to 14 mm. below the former systolic pressure. The extent of the fall in blood pressure depended upon the compression used, the most marked change occurring with the highest compression pressure.

In all of the experiments described above in which blood pressure records were made the pressure exerted on the brachial artery was a little below *systolic*. If the changes in amplitude observed are due to changes in blood pressure, we should expect, when the pressure in the sphygmomanometer is set at a level slightly lower than *diastolic*, compression and decompression to produce changes in the opposite direction to those observed in the records thus far described. For in that case a rise of the systolic pressure, the pressure exerted on the brachial artery remaining constant, would increase the difference between the systolic pressure and the pressure exerted by the sphygmomanometric cuff, and the oscillations would diminish in amplitude (fig. 3). Conversely, a fall in the systolic blood pressure would be indicated by an increase in amplitude. Such a reversal in the effects would not occur if the changes in the amplitude of the oscillations were due to pulse pressure changes or to extraneous movements of the body.

The records made with this low pressure in the sphygmomanometer (60 to 75 mm. Hg.) showed very often a decrease in the amplitude of the oscillations upon applying the pressure to the legs, but never an increase. On releasing the compression, an increase was always observed, indicating that the effects were in reality due to changes in blood pressure (fig. 4).

Thus there can be no doubt but that the changes in amplitude of oscillations noted in the continuous blood pressure record actually indicate changes in blood pressure. Reading the changes of amplitude of oscillations in terms of blood pressure, it may therefore be concluded that compression of the thighs raises the arterial blood pressure, while decompression has the opposite effect. This effect is the more pronounced the greater the compression pressure used.

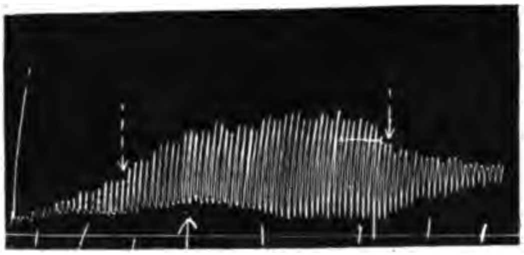

Fig. 3. Sphygmomanometric record to illustrate the principle of reversal. Arrows indicate pressure slightly below systolic, and slightly below diastolic, respectively.

Cause of the blood pressure changes. The fact that the changes in oscillation amplitude were due to alterations of blood pressure having been established, the immediate cause of these blood pressure changes was next sought. Several factors had to be taken into consideration in this connection. In the first place, the blood pressure might be altered by vasomotor reflexes due to stimulation of the skin by the sudden application and removal of pressure through the cuffs. Again, the blocking of the venous flow which takes place particularly at the lower and medium pressures, with its storing up of blood in the legs and the subsequent infusion of this blood into the inferior vena cava when the pressure is released might have an effect on the general arterial pressure. Alterations in blood pressure might also result from changes in the caliber of the arteries and hence in the resistance to the flow of blood through them brought about by compression and decompression.

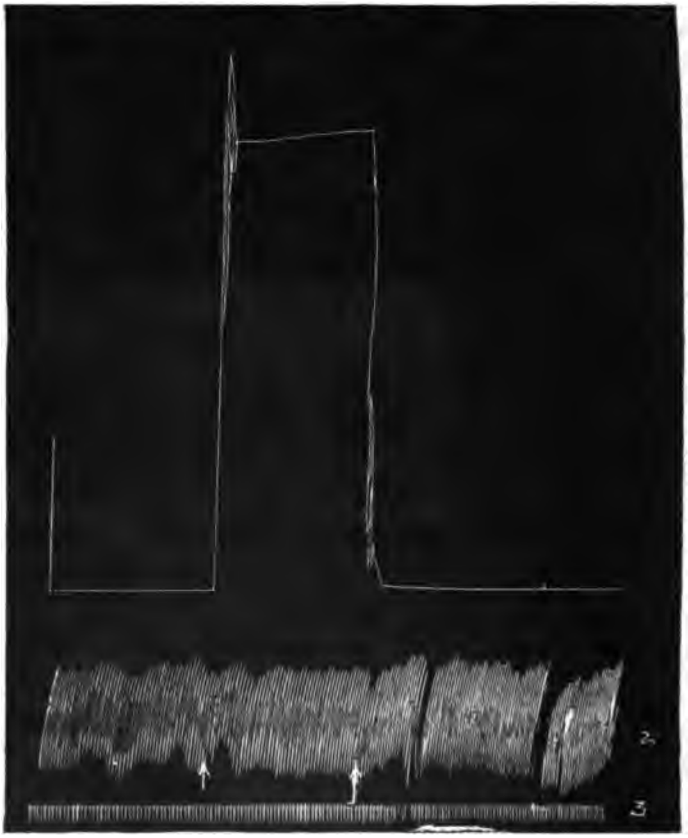

Fig. 4. Record illustrating reversal. *1*, Compression pressure (75 mm. Hg.); *2*, sphygmomanometric record from left arm; *3*, time in seconds.

To show that sensory stimulation and reflexes through the vasomotor centers effected thereby were not factors, attempts were made to supply the same sensory effects while protecting the femoral artery and vein from compression. This was accomplished by protecting the blood vessels of the thigh from compression by placing beneath the cuff a piece of stiff wire netting bent so as to form a bridge over the vessels. That the flow through the thigh vessels was by this means preserved practically at the normal level was indicated by the fact that there was little or no increase in the volume of the legs when the compressing pressure was thrown into the thigh. Changes in amplitude of the oscillations in the sphygmomanometer record were slight or altogether absent, whereas in the records made with the same pressures but without the protection, the changes were considerable.

The effect of removal of blood from and addition of blood to the general circulation was investigated by means of the volume recorder which was connected with the plethysmograph. A compression of 10 to 20 mm. had no effect on the volume of the leg, excepting in one determination where a very slight accumulation of blood was noted. Usually accumulation of blood began to occur in the leg at 30 mm. Hg.[1]

The increase in volume of the leg consequent to compression is slow at low compression but the rate increases with the compressing pressure until it reaches approximately 80 mm. Hg. It then remains fairly constant until 110 mm. is reached. Above this pressure a rapid diminution in the rate of increase takes place. The total volume increase is greatest at 70 to 110 mm. pressure. Above 110 mm. the rate of accumulation of the blood decreased very rapidly with increasing compression, and at 140 mm. the flow of blood through the arteries was completely blocked. It is thus seen that the blocking of the arterial flow in the leg did not become complete until the compression pressure had risen 10 to 20 mm. above the systolic pressure as determined in the arm (see table 1 and fig. 9, p. 295). This is probably due in part at least, to the resistance of the tissues and the imperfect transmission of the compressing pressure through them.

The plethysmograph gave information with regard to volume changes in the foot and lower leg. Inasmuch as the volume of both legs from the position of the cuffs down is changing it therefore seems justifiable to assume that the total volume change is roughly four times the amount indicated by the recorder. The recorder had a maximum capacity of 40 cc. In many of the observations this capacity was exceeded. In such instances we are justified in assuming that the total accumulation of blood in the legs exceeded 160 cc.

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[1] The increase in volume indicated by the plethysmograph under the influence of low compressing pressures is probably much too small, for the band tubing employed for the purpose of closing the plethysmograph exerts a certain amount of pressure on the leg. This will cause the veins in the part of the leg within the plethysmograph to fill with blood to the pressure exerted by the band tubing when a balance will be struck between inflow and outflow. This balance will be disturbed by compression of the thigh vessels practically only when this compression raises the venous pressure in the part of the leg without the plethysmograph above the venous pressure in the plethysmograph.

The decrease in volume of the leg with decompression is very rapid. Never has more than twelve seconds been required for the leg to attain its initial volume, and usually by far the greatest part of the blood has left the leg within five or six pulse beats.

It may be assumed for reasons given above that decompression at low compressing pressures lets into the circulation a very much larger volume of blood than the figures in the table indicate. Since at very low compressing pressures (10 to 40 mm. Hg.) interference with arterial flow is certainly a negligible factor and since decompression at those pressures causes no or very slight alterations of arterial pressure it may be concluded that the addition to the general circulation of more than 40 cc. of blood in the course of two or three seconds is handled without affecting the general blood pressure.

Mention was made above of the fact that compression pressures of 40 to 50 mm. Hg. had given no increase of the oscillations on the sphygmomanometric record. Likewise several estimations made by the auscultatory method failed to show any fall in the pressure resulting from occlusion of the veins alone. It would therefore seem that in man the withdrawal of more than 64 cc. of blood as in this experiment does not affect the general blood pressure. It must be remarked, however, that these pressures are not those at which the maximum accumulation of blood in the legs takes place.

Whether the removal of the much larger volume of blood that occurs at somewhat higher compression pressures causes a fall of blood pressure we have not been able to determine because of the complication that results from the partial occlusion of the artery that then takes place.

Effect of low compression pressures on the caliber of arteries. If the conclusion is correct that the rise in general arterial pressure consequent upon the application of compression to the thighs is the result of an increase in arterial resistance, it follows that such low compression pressures as 50 or 60 mm. Hg. must diminish appreciably the caliber of the femoral arteries, otherwise the pressure changes determined by these slight compressions could not be explained on the basis of arterial resistance.

That low compressing pressures materially reduce the flow through an artery was shown by a direct experiment in which a dog's carotid artery inserted in a circulation scheme was placed in a compression chamber by means of which any desired pressure could be exerted on the artery. Under a head of 66 mm. Hg. salt solution was allowed to flow through the artery as the compression pressure was lowered in steps from 68 mm. Hg. to zero. The results on the rate of flow through the artery are shown in table 2.

If we assume that the diastolic pressure in man is as in this experiment 66 mm. Hg., it is obvious that even so slight a compression as 15 mm. Hg. may result in a 22 per cent reduction in the diastolic flow, but only, of course, when the peripheral resistance is very low. It is also obvious that, owing to the diminution in the extensibility of the artery with increasing distention, the effects of compression will diminish with the increase in arterial pressure. As the result of this experiment we believe that the application of pressures less than diastolic to a limb may, under certain circumstances have a considerable effect upon the caliber of the artery of that limb and therefore upon the resistance to the flow of blood through the artery. This fact may have some bearing on the Hewlett method of determining the velocity of the blood flow in man.

TABLE 2

Flow through the artery at different compression pressures

Pressure head = 66 mm. Hg.

TRIAL NO.	COMPRESSION IN MM. HG.	FLOW IN CC. PER SECOND	CONDITION OF ARTERY
1	68	0.0	Artery closed
2	63	0.38	Artery open
3	58	1.00	Artery open
4	54	1.5	Artery open wider
5	41	3.4	Artery open wider
6	28	4.96	Artery open still wider
7	15	6.92	Artery open still wider
8	6	8.08	Artery almost full
9	0	8.84	Artery wide open

Attempt to dissociate arterial from venous effects. The method described above having failed to show any marked changes in blood pressure resulting from occlusion of the veins alone, the attempt was made to differentiate arterial resistance effects from those due to the outflow of accumulated blood from the legs during decompression. A high compressing pressure, 140 mm. Hg., under which no blood accumulated in the leg, was applied for fifteen seconds, then released and the effect noted. Now a low pressure, 70 mm. Hg., was applied for thirty seconds with its consequent damming back of blood in the legs, and was immediately followed by a high pressure, 140 mm. Hg., for fifteen seconds. This pressure was then released suddenly so as to produce the first effect plus an outflow of accumulated blood. The first procedure was then repeated. These experiments were repeated several times, varying the time intervals. No characteristic difference could be observed between the effect of a high compression alone and that of the same compression for the same length of time preceded by a low compression. These experiments seem to confirm the conclusion reached by the analysis of preceding results that the accumulation of blood in the legs and its sudden return to the circulation is not an important factor in the circulatory phenomena of compression and decompression of arteries and veins.

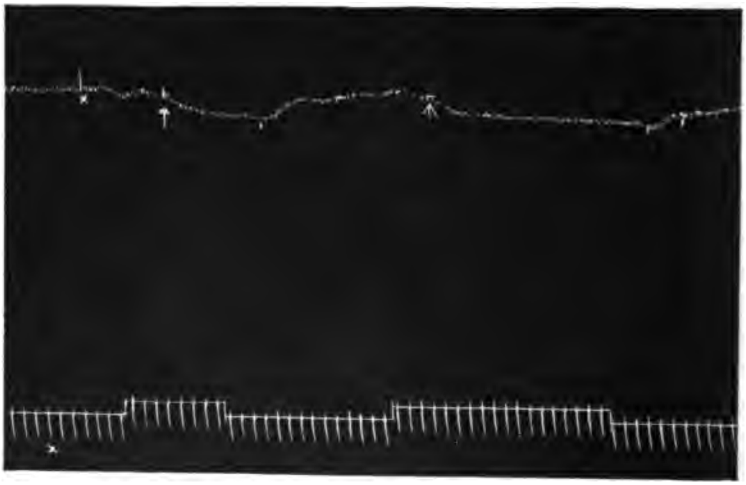

Fig. 5. Effect on arterial blood pressure of occlusion of inferior vena cava (cannula in carotid). Arrows indicate moment of occlusion.

Results with animals An experiment was then performed on a rabbit for the purpose of determining the effect of these or at least similar procedures in animals. The abdomen was opened under ether anaesthesia, a ligature was passed around the inferior vena cava just above the junction of the common iliac veins, another around the abdominal aorta, and a third around both vessels. The two ends of each ligature were drawn through a glass tube so that by pulling upon the projecting ends the vessel could be conveniently occluded. Upon compression of the vena cava the arterial pressure was observed to fall 6 mm. during the compression, somewhat more rapidly at first. Upon decompression the arterial pressure returned somewhat more rapidly to normal (fig. 5). Occlusion of the abdominal aorta caused the blood pressure to mount at first rapidly, then more slowly. The total rise was 10 mm. Hg. On decompression, the blood pressure fell rapidly to a point below normal and then slowly returned to normal (fig. 6). Compression of both vessels together caused an effect resembling that due to compression of the aorta alone. It differed from it slightly in that there was a small rapid rise in pressure followed by a fall, then a slow rise until decompression, which was followed by a fall in the blood pressure, a little slower than that due to aortic decompression, to a point below normal, then a slow return to normal (fig. 7). The total rise in this latter case amounted to 6 mm. Hg. The results obtained upon compression of the aorta alone and of the aorta and vena cava together are similar to those obtained upon aortic compression by Colson (2) in the dog and by Sollmann and Pilcher (3) in the dog and cat.

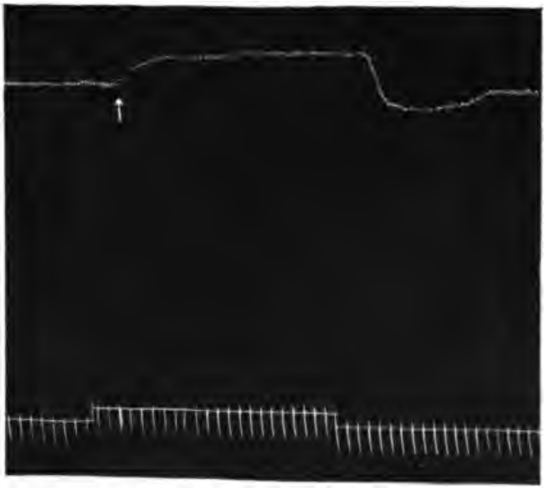

Fig. 6. Effect on arterial blood pressure of occlusion of abdominal aorta. Arrow indicates moment of occlusion.

Considered in the light of this experiment, the effects observed in man can be explained in the main as due to changes in arterial resistance. They may also have been affected, to some extent, by the blocking of the venous flow, the accumulation of blood in the legs, and its subsequent discharge into the circulation. Thus the delayed rise of pressure on compression with the lower pressures may as in animals be due to venous obstruction, and the smaller fall in pressure due to decompression may be the result of the entrance of the accumulated blood into the system from the legs.

Effect of compression and decompression upon the heart rate. To determine the effect of compression and decompression upon the rate of the heart, 35 records were examined. The compression pressures used in these records ranged between 55 and 130 mm. Hg. Ten different periods consisting of five pulses each were examined in each record. These periods were so selected that the rate of the heart could be calculated immediately before the application of pressure, immediately afterward, midway between the application and release of the pressure, immediately before and after release of pressure, and at the end of the record. The heart rate was determined by counting the number of seconds in which the group of five pulse beats occurred.

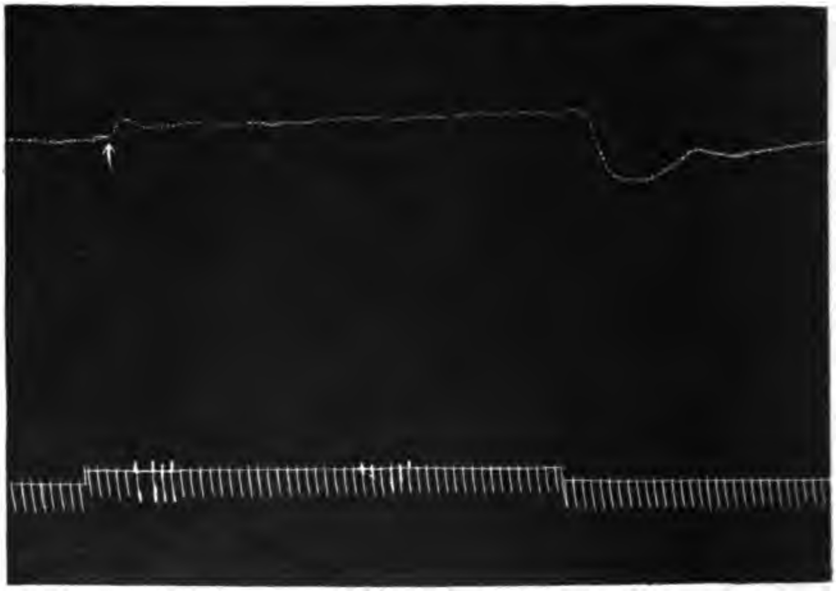

Fig. 7. Effect on arterial blood pressure of occlusion of both abdominal aorta and inferior vena cava. Arrow indicates time of occlusion.

Compression was followed by slowing of the heart rate in 55 per cent of the cases, no change in 25 per cent and some acceleration in 20 per cent. Decompression was followed by acceleration of the heart rate in 60 per cent of the cases, no change in 20 per cent, and slowing in 20 per cent.

Inasmuch as the rate must be calculated for very short periods, not over five seconds, it is obvious that not infrequently such changes in heart rate as may be caused by compression and decompression will be obscured by such concurrent changes in heart rate as occur in association with respiration, deglutition and similar processes. It is not surprising that the effect of compression and decompression on the heart rate is seemingly not always the same. The figures obtained therefore leave little room for doubting that compression retards and decompression accelerates the heart rate.

A few records were made with the kymograph moving at such a rate as to make possible the determination of the duration of each individual pulse. The results obtained in this way are essentially the same as those just mentioned. In addition, they show that the changes in the duration of the cardiac cycle follow immediately upon the changes of compression pressure.

The slowing of the heart beat upon compression can hardly be ascribed to anything but the rise of arterial pressure due to the increase in the arterial resistance. The acceleration observed following decompression can most satisfactorily be explained as due to the sudden fall of arterial pressure due to diminution in peripheral resistance, although the flow of the blood accumulated in the legs into the central venous system might conceivably play a part, especially as Bainbridge (4) has shown that the injection of blood or salt solution into the circulation of dogs, raising the venous pressure, is associated with acceleration of the heart, due to the increased venous pressure.

Observations on a case of femoral arterio-venous aneurysm. The opportunity presented itself of making some observations on a case of arterio-venous aneurysm in the Barnes Hospital which have a very direct bearing on the questions this paper deals with. A communication existed between the right femoral artery and vein. The effect of closing and opening the communication on the arterial pressure was determined. This was done by applying pressure over the site of the communication with the fingers while a continuous blood pressure record was being made with the Erlanger sphygmomanometer. The effect of closure came on within one or two heart beats and consisted in a tremendous rise of blood pressure and a slowing of the pulse rate (fig. 8). The blood pressure as determined by the auscultatory method rose in one experiment from 128 to 180 mm. Hg. Opening the communication caused a fall in blood pressure and an acceleration of the heart and restored the original conditions.

It is evident that in closing the communication we are shutting off the easy path of the blood into the veins and hence back to the heart, thus relieving the heart and indeed the entire venous system of an abnormally high pressure. At the same time the peripheral resistance is greatly increased. Opening the communication, of course, has the opposite effects. It seems fair to conclude that the changes in blood pressure here observed must be due to the changes in arterial resistance, as there seems to be no reason for believing that reducing the venous pressure and, for a time at least, the volume of blood supplied to the heart would cause the arterial pressure to rise (5).

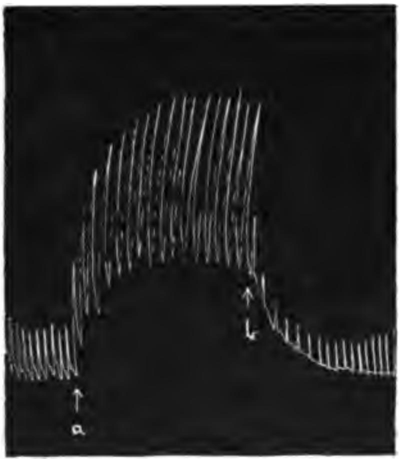

Fig. 8. Sphygmomanometric record from case of femoral arterio-venous aneurysm during closure and opening of the communication. Closed at *a* and opened at *b*.

The slowing of the heart rate observed on closing the arterio-venous opening was probably due to stimulation of the vagus center by the increased pressure, for at the operation after the patient had received atropine sulphate, there was no marked change in rate when the communication between femoral artery and vein was ligated. Some time after the operation, however, the heart rate slowed down to about 70 beats per minute, having been 95 per minute before it.

The phenomena observed in this case agree with those observed in the experiments in man and also in the rabbit. They may all be explained in the main, at least, on the basis of changes in arterial resistance.

Compression of the arm. In order to determine whether or not compression of the arm as in making blood pressure determinations affects the general blood pressure, the cuff of the sphygmomanometer was placed on the left arm and a continuous blood pressure tracing made while different pressures were applied to the right arm through another similar cuff. Pressures of 60 to 140 mm. Hg. were employed. No effects were observed. The results were such as to indicate that the changes in blood pressure resulting from the application of such a cuff to one arm, if they exist, are negligible.

SUMMARY

The effect of the occlusion of blood vessels upon the general blood pressure has been studied in man.

Circular compression of the thighs causes a rise in blood pressure, larger and more immediate at the higher than at the lower compressions. This is due to the increase in arterial resistance, modified somewhat at the lower compression pressures by the venous effect. The heart rate is somewhat slowed.

Decompression of the thighs causes a fall in blood pressure, more immediate and more profound than the rise due to compression. This effect is likewise more marked at the higher compression pressures and is modified slightly by the venous accumulation in the legs. The heart rate is somewhat accelerated.

The removal from the circulation of man of 64 cc. of blood does not change appreciably the arterial pressure.

A slight external pressure, even less than the diastolic pressure, upon an artery may under certain circumstances materially diminish the flow through it.

These results are in agreement with those obtained in the rabbit in so far as the arterial pressure is concerned, and in so far as it is possible to duplicate in man the conditions of an animal experiment.

The results of the experiments in man and in the rabbit in so far as the arterial blood pressure is concerned, are borne out by some observations on a case of femoral arterio-venous aneurysm.

The work was undertaken at the suggestion of Dr. Joseph Erlanger, and I wish to express my indebtedness to him for his advice and criticism during the preparation of this paper.

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THE EFFECT OF ROTATION AND OF UNILATERAL REMOVAL OF THE OTIC LABYRINTH ON THE EQUILIBRIUM AND OCULAR REACTIONS IN KITTENS

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The ocular and other phenomena which present themselves after unilateral extirpation of the otic labyrinth in the adult cat and dog have been worked out in considerable detail by Wilson and Pike (1). These authors have found that immediately after recovery from the anesthetic, there is deviation of the eyes toward the side of the lesion, accompanied by nystagmus. This nystagmus, which is due in great part to the unbalanced activity of the intact labyrinth, consists of two phases: a slow deviation of the eyes to the side of the lesion, followed by a quick return to the initial position.

These ocular movements do not differ appreciably from those observed when the head is rotated in space and can be especially well studied in the post-rotatory period, in man as well as in certain other mammals. With the head in the vertex upward position there is a relatively slow deviation of the eyes in the direction of the preceding rotation with a quick return to the midline. In an extensive study of labyrinthine ocular movements in man, Bartels and Ziba (2) found that in the sleeping newborn and in prematurely born children (up to the seventh or eighth month) rotation of the head in space does not produce nystagmus. There is, however, a deviation of the eyes opposite to the direction of rotation during the rotatory period. On cessation of rotation the eyes return slowly to the position of rest.

As the study of the ocular movements following unilateral removal of the labyrinth has been confined to fully grown mammals, the observations of Bartels and Ziba at once suggest the study of these eye movements in very young animals under the same experimental conditions.

Method. The experiments were performed on kittens ranging in age from six days to seven weeks. The labyrinth was removed under aseptic conditions according to the method described by Wilson and Pike (1). Great care was taken not to injure the cerebellum or cerebrum and cases showing such injury were eliminated from the series. In all cases the labyrinthine operation was preceded by rotation experiments. The animals were rotated about their ventro-dorsal axis by means of a turn table.

Experiment I. Kitten six days old

Palpebral membranes intact, but tore readily on forcible separation of the eyelids. Eyes midway between normal and embryonic position, i.e., with axes of vision markedly divergent. The animal crawls about unsteadily with limbs outstretched and with claws extended. When placed on its side, the animal returns to the standing position slowly and with apparent difficulty.

During the rotatory period, the head moves slightly opposite to the direction of rotation. In the post rotatory period, the head returns slowly to the mid line. No perceptible deviation of the eyes. No nystagmus.

Right otic labyrinth removed under ether. Slight torsion of the head with occiput to right. No apparent changes in equilibration. No appreciable change in the position of the eyes. No nystagmus. This animal was kept under observation for 36 hours. No changes were noted during this period. Death on the second day after the operation. Death in this case is attributed to exposure and inanition, as the mother avoided and refused to nurse the kitten following the operation. No signs of infection about operative wound.

Experiment II. Kitten ten days old

Eyes open for the last 24 hours. Walks about unsteadily with limbs outstretched, otherwise equilibration fair.

During the rotatory period the head moves opposite to the direction of rotation. During the post rotatory period the head moves in the direction of the preceding rotation for a few seconds, and then returns to the mid line with slow oscillatory movements. The eyes oscillate irregularly in a position of deviation in the direction of the preceding rotation. This effect is temporary, the eyes returning slowly to the position of rest. These oscillations do not present the alternating slow and rapid phases of well developed labyrinthine nystagmus.

(Another kitten 10 days old from the same litter, presented a slow post-rotatory nystagmus, about 6 cycles per minute, with the slow phase in the direction of the preceding rotation. The quick phase was not well marked, being only slightly more rapid than the slow phase. The head reactions did not differ in any respect.)

Left otic labyrinth removed. On recovery from the anesthetic there is marked torsion of the head (occiput to left). Practically no disturbances of equilibrium. The eyes deviate to the side of the lesion (fig. 1). No oscillation or nystagmus. At the end of 48 hours, position of head unchanged. Eyes still deviate to side of lesion, although the right pupil has shifted slightly toward the mid line. On the third day following the operation, this kitten was devoured by its mother.

Experiment III. Kitten two weeks old

Weight 220 grams. Slight unsteadiness of gait. In the post-rotatory period the head is carried in the direction of preceding rotation for a few seconds and then returns to the mid line. Irregular oscillations of the eyes, for a few seconds, in a position of slight deviation in direction of preceding rotation.

June 1, 1916, 9.55 a.m. Left labyrinth removed.

10.20 a.m. Marked torsion of head, occiput pointing to left. Animal shows tendency to fall to left side in walking. Eyes deviate to left and down (fig. 2), but no nystagmus.

10.30 a.m. At rare intervals slight oscillations of the eyes but no distinct nystagmus. Lateral nodding movements of head, slow to left, more rapid to right.

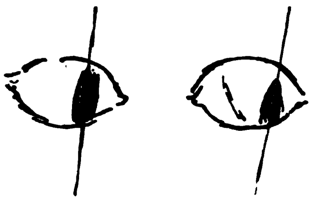

Fig. 1

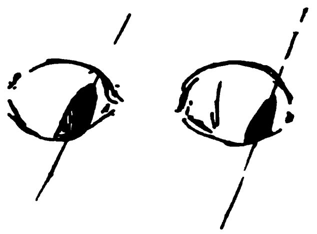

Fig. 2

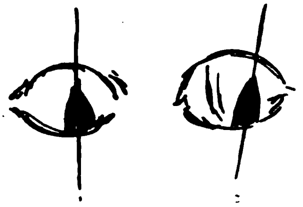

Fig. 3

June 2. Eye positions unchanged. No oscillations or nystagmus. Torsion of head as before. Equilibrium disturbances have disappeared to a great extent. When placed on its left side, the animal experiences difficulty in regaining the standing position. This is not the case when it is placed on its right side.

June 4. Equilibrium disturbances absent. Torsion of head unchanged. No ocular movements. Deviation greatly diminished, especially of the right eye (fig. 3).

June 5. Ocular deviation has entirely disappeared. Torsion of head as marked as before.

June 7. No change in behavior of animal since June 5.

Experiment IV. Kitten three weeks old.

Weight 275 grams. During rotation animal turns about its dorso-ventral axis away from the direction of rotation. The neck also bends in the same direction. During the post rotatory period, there is turning of the body about its dorso-ventral axis and bending of the neck in the direction of preceding rotation. Slow post-rotatory nystagmus appears after a latent period. Slow phase in direction of preceding rotation and quick return in the opposite direction.

August 28, 1916, 10 a.m. Left labyrinth removed.

2 p.m. Animal shows tendency to turn about its dorso-ventral axis toward the side of the lesion. Occasionally falls in the same direction. Coarse vertical tremor of head. Marked torsion of head, occiput pointing toward the side of the lesion. Deviation of eyes in the direction of the lesion. At rare intervals slow oscillations of eyes or doubtful very weak nystagmus. When these movements appear the eyes oscillate about once every ten seconds.

August 29. Disturbances of equilibrium have entirely disappeared. Eye on the intact side shows slight decrease of deviation.

August 31. Deviation of eyes unchanged since last note. No ocular oscillations.

September 9. Observations since August 31 show progresssive diminution of deviation.

September 11. Deviation has entirely disappeared. Torsion of head unchanged.

Experiment V. Kitten three weeks old

Weight 266 grams. Animal shows distinct but slow post-rotatory nystagmus and the same general reactions as observed in kitten of Experiment IV.

June 25, 1916, 3 p.m. Right labyrinth removed. On recovery from the anesthetic the animal presents marked torsion of the head, occiput pointing in the direction of lesion. Marked deviation of eyes to side of lesion with very slow but distinct and irregular nystagmus.

June 26. Torsion of head unchanged. Deviation of eyes as before with slow nystagmus. The latter is often interrupted, the eyes remaining in the deviated position. Slight locomotor disturbances.

June 28. Locomotor disturbances have entirely disappeared. Torsion of head unchanged. Deviation of eyes not as marked as at last observation. Nystagmus has entirely disappeared. The eyes follow moving objects readily to the right but not to the left. There is absolute inability to turn the eyes past the mid line in the direction opposite to the operated side.

June 29. Deviation of eyes has entirely disappeared, but inability to turn the eyes past the mid line to left remains.

Experiment VI. Kitten three weeks old

Weight 283 grams. Rotation tests elicit the same symptoms described under Experiments IV and V.

June 25, 1916. Right labyrinth removed. Torsion of head to right. Nystagmus and deviation as in Experiment V. This animal however, showed practically no equilibrium disturbances. Lateral oscillations of the head occur at intervals of about five seconds. These head movements are slow toward the lesion but rapid and violent in the opposite direction.

June 26. Torsion of head unchanged. Deviation of eyes to side of lesion with occasional appearance of nystagmus at irregular intervals. The eyes move slowly to the side of the lesion and return to the mid line distinctly faster. During the quiescent period the eyes remain in the position of deviation. Oscillations of head now occur at rare intervals.

From the 26th to the 29th of June this animal displayed exactly the same symptoms as the kitten of Experiment V during the corresponding dates.

Experiment VII. Kitten four weeks old

Usual responses to rotation. Post rotatory nystagmus of moderate speed. As in the kitten of three weeks there is a distinct latent period, unusually marked in this case, before the appearance of nystagmus. This latency will be referred to later.

September 4, 1916, 10 a.m. Left labyrinth removed.

1 p.m. Marked torsion of head and bending of neck to the side of the lesion. Occasional rapid oscillations of the head in the same direction, with slow return to the mid line. The animal moves about without difficulty, although there is a tendency to walk in a curve toward the left. Deviation of the eyes to the side of the lesion with slow but very distinct nystagmus (four to six oscillations per minute).

September 5. Very slight tendency to turn to the left in walking remains. Torsion of the head as before. Slow nystagmus present (about five oscillations per minute). The eyes move rather slowly to the mid line and after a few seconds return very slowly to the position of greatest deviation.

September 6. Nystagmus has entirely disappeared. Otherwise, no change since last note.

September 9. Deviation of the eyes has diminished gradually since September 8. The eyes are now in the mid line position, but there is apparent inability to turn the eyes to the extreme right.

Experiment VIII. Kitten five weeks old

Post rotatory motor disturbances and nystagmus well marked. The latter is rapid and occurs *immediately* after cessation of rotation.

September 11, 1916, 5 p.m. Left labyrinth removed.

September 12, 8 a.m. Marked torsion of the head to the side of the lesion. Animal turns to the left in walking. Oscillations of the head similar to those seen in the kitten of Experiment VII. Deviation of the eyes to the side of the lesion and nystagmus well marked. Rate of nystagmus: 15 per minute.

5 p.m. Nystagmus appears at irregular intervals. Rate unchanged during active periods. Deviation of the eyes unchanged.

September 13. Very slight deviation of the eyes remains. No nystagmus. Torsion of head unchanged.

September 14. Deviation of the eyes has entirely disappeared. There is, however, inability to turn the eyes past the mid line in the direction opposite to the side of the lesion. Torsion of the head unchanged.

September 15. No change since last note.

Experiment IX. Kitten seven weeks old

Reactions to rotation as seen in full grown cats. Rapid nystagmus and violent compensatory movements.

June 1, 1916, 5 p.m. Left labyrinth removed. Immediately after recovery from the anesthetic, torsion of the head to the side of the lesion appears. Deviation of the eyes to the side of the lesion with very rapid typical labyrinthine nystagmus. Tendency to turn to the left side in walking. Occasionally falls to the left. The head is carried violently to the right and then returns to the extreme left by a series of rapid oscillations.

June 2. The nystagmus has diminished in rate and is now interrupted by quiescent periods when only slight deviation remains. When the animal's attention is fixed, nystagmus is absent for a period of 40 to 120 seconds. Otherwise, the nystagmus movements occur in groups at intervals of about 10 seconds.

June 3. Nystagmus now occurs at infrequent intervals. Deviation of the eyes has almost disappeared. The animal seems unable to turn the eyes to the extreme right, i.e., in the direction opposite to the labyrinthine lesion. Oscillations of the head are now infrequent, and much less violent in character.

June 4. Deviation of the eyes, nystagmus and oscillations of the head have disappeared.

This animal was kept under observation for almost two months (until July 28). The inability to turn the eyes to the extreme right and the torsional effect on the head were the only persisting symptoms, as noted in several other animals. The torsion of the head in the direction of the lesion has been shown to result from loss of tonus in the cervical musculature on the side of the lesion (3). In view of this fact, the inability to turn the eyes to the canthi opposite to the side of the labyrinthine lesion, suggests the possibility of an asthenia in the recti muscles contralateral to the injury.

SUMMARY

Rotation experiments.

The reactions observed during the rotatory and post-rotatory periods increase, up to a certain limit, proportionally to the age of the animals. Kittens up to the second week after birth present only head and neck movements. After the third week the animal reacts bodily, turning on its ventro-dorsal axis. These general reactions increase progressively until the seventh week, at which time the behavior does not differ appreciably from that of the fully grown cat. As a whole, these reactions seem to progress *pari passu* with the development of equilibration.

Progressive changes are also seen in the ocular reactions. During the first two weeks after birth, the post-rotatory nystagmus is either absent, or when present, of a slow and irregular type. At the third week well defined labyrinthine nystagmus first becomes evident, the slow and rapid phases being distinguished without difficulty. The rate of nystagmus, slow in the younger animals, increases progressively until the fifth to the seventh week, when it does not materially differ from the post-rotatory nystagmus of the fully grown animal.

In animals of three to four weeks, the post-rotatory nystagmus is preceded by a latent period. For a few seconds after cessation of rotation the eyes, fixed in the median position, present a fine and very rapid horizontal tremor. The onset of nystagmus then occurs suddenly, the transition being extremely striking.

The following table shows the results obtained with a full grown cat, a five-week-old kitten, and one of four weeks, in which this latency was unusually pronounced. The animals were suspended by a string tied just below the fore limbs, and rotated at approximately the same speed. It will be noted that the latent period is absent in the older animals.

TABLE 1

	DIRECTION OF ROTATION	PERIOD OF ROTATION	LATENCY	DURATION OF NYSTAGMUS
		seconds	*seconds*	*second*
Kitten, Experiment VII, four weeks old........	To left	7	7	9
	To right	8	6	10
	To left	7	8	10
	To right	8	8	10
Kitten, Experiment VIII, five weeks old........	To right	6	0	8
	To left	9	0	19
	To right	8	0	23
	To left	10	0	18
Full grown cat................................	To right	9	0	9
	To left	8	0	7
	To right	8	0	8
	To left	10	0	9

The effects of unilateral removal of the otic labyrinth

Torsion of head. With the exception of the six-day-old kitten in which the torsion of the head to the side of the lesion was slight, all the animals of the series presented torsion which did not differ from that seen in the full grown animals. This torsional effect, as shown by Wilson and Pike (1) is permanent. The kitten of Experiment IX, kept under observation for two months, showed no appreciable change in the posture of the head. As this torsion is due to impaired tonus of the cervical musculature on the side of the lesion (3), the absence of marked torsion in the six-day-kitten may be attributed to late myelination of the labyrinthine tonus paths.

Equilibrium. The general disturbances of equilibrium following removal of the labyrinth, as in the rotation experiments, increase progressively with the age of the animals. As a whole the intensity of these symptoms is proportional to the developmental stage of the function of equilibration. Up to the second week after birth, these reactions are confined mainly to movements of the head. From the second week on equilibration disturbances of the body appear, which become progressively more violent until between the fifth and the seventh week, when these reactions are identical to those obtained in the fully grown cat. The equilibrium disturbances diminish in intensity after the first twenty-four hours and have entirely disappeared at the end of the second or third day.

Ocular movements. With the exception of the six-day kitten, in which no change in the position of the eyes was discernible, there is up to the end of the second week after birth deviation of the eyes toward the side of the lesion without distinct nystagmus. During the second week after birth, slight irregular oscillations of the eyes occur at intervals, but the fully developed two phase nystagmus does not appear until the third week. The nystagmus as seen in the three-week-old animals is of a slow type and interrupted. From the fourth week on it becomes regular. In the younger animals the oscillations occur at the rate of 4 to 6 per minute. This rate increases progressively, so that at the seventh week the rapidity of nystagmus approximates that seen in the fully grown animal.

In animals of three to five weeks, the nystagmus diminishes in intensity during the first twenty-four hours after the operation, and has entirely disappeared between the twenty-fourth and thirty-sixth hours. The deviation of the eyes on the other hand, does not begin to decrease appreciably until about the end of the forty-eighth hour, and persists for three to five days (in one case for ten days). In the seven-week-old kitten, as in fully grown cats, the deviation and the nystagmus disappear simultaneously.

If the ocular reactions following unilateral removal of the labryinth are compared with those observed in the rotation experiments, it will be seen that definite post-rotatory nystagmus occurs at an age (ten days to two weeks) when post-operative nystagmus does not appear. According to the views of Wilson and Pike (1) the nystagmus resulting from labyrinthine extirpation is due in great part to the unbalanced activity of the intact labyrinth. This difference in reaction, therefore, seems to indicate that the stimulus derived from rotation is much more effective in producing compensatory eye movements.

Without wishing to emphasize the ontophylogenetic importance of some of the ocular reactions observed, the relative persistence of deviation and the absence of nystagmus in the younger animals seem to me suggestive, in view of the following facts:

1. The intensity of nystagmus, after unilateral removal of the labyrinth, varies as a rule with the position of the animal in the vertebrate phylum. While absent in the lower forms, its intensity is greatest in the higher mammals.

2. In the turtle, unilateral destruction of the labyrinth is followed by permanent deviation of the head to the side of the lesion (unpublished experiments by Professor Pike).

3. In prematurely born children rotation of the head in space is accompanied by deviation of the eyes *without nystagmus*. Observations of Bartels and Ziba (2).

Wilson and Pike (1) have shown that labyrinthine nystagmus is dependent on the integrity of two nervous paths, one associated with deviation, the other with the quick return. These authors have shown that the quick phase of nystagmus, after labyrinthine removal, can be eliminated by *complete* decerebration, including the optic thalamus. After this procedure deviation of the eyes alone remains. In another article the same authors (4) basing their conclusions on the work of Tozer and Sherrington (5) suggest that, "in labyrinthine nystagmus, the abnormal deviation of the eyes sets up sensory impulses in the afferent endings of the eye muscles themselves which bring about a return, either reflex or voluntary, of the eyes to the normal position. Nystagmus would then be a response to kinesthetic sensations." Accordingly, the absence or the undeveloped character of the quick phase, in the very young animals, may be explained by a relatively late myelination either of the kinesthetic paths related to the cerebrum and thalamus or of the efferent paths conveying impulses from the cerebrum and thalamus to the ocular muscles.

CONCLUSIONS

1. In kittens, the general motor reactions which follow rotation and unilateral removal of the otic labyrinth are, up to a certain limit, proportional to the age of the animals. As a whole these reactions

seem to progress *pari passu* with the development of the function of equilibration.

2. The ocular reactions, likewise, show progressive changes. The compensatory eye movements following rotation of the head in space are at first of a slow and irregular type, but well defined labyrinthine nystagmus does not appear until about the third week after birth. The rate of post-rotatory nystagmus increases gradually with age.

3. In the younger animals post-rotatory nystagmus is preceded by a latent period.

4. Following unilateral removal of the otic labyrinth there is, up to the end of the second week after birth, deviation of the eyes to the side of the lesion without distinct nystagmus. At three weeks after birth nystagmus, of an intermittent type and of a slow rate, appears. The rate and regularity of the post-operative nystagmus, as in the rotation experiments, increases progressively with the age of animals.

5. In animals of three to five weeks the post-operative nystagmus disappears relatively early, whereas the deviation of the eyes to the side of the lesion persists for several days. In older animals the nystagmus and deviation disappear simultaneously.

6. The probable ontophylogenetic importance of these ocular reactions is suggested.

7. The ocular and other phenomena observed following rotation and unilateral removal of the otic labyrinth are explained by a relatively late myelination of the nervous paths concerned.

I wish to take this opportunity to thank Professor Pike for his kind assistance in the development of the operative technique used in these experiments.

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STUDIES ON BIOLUMINESCENCE

IV. THE CHEMISTRY OF LIGHT PRODUCTION IN A JAPANESE OSTRACOD CRUSTACEAN, *Cypridina hilgendorfii*, MÜLLER

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INTRODUCTION

While a large and ever increasing number of organisms are known to produce light, in only a few of these is there sufficient light-giving substance to make chemical studies feasible. Luminous bacteria (1) are of practically no value, as also *Noctiluca* (2) and luminous squid; the fire-fly (3) and a pennatulid, *Cavernularia* (4) are somewhat better, while the mollusc, *Pholas dactylus* (5), judging from Dubois' results, is better still. By far the most valuable of any of these organisms, if I may make comparisons without actual experience with *Pholas dactylus*, is the small crustacean, *Cypridina hilgendorfii*. For the size of animal, the light-giving substance is relatively enormous and its light-giving power incredibly great. Suffice it to say that one part of luminous gland substance in 1,600,000,000 parts of water will give visible light under proper conditions. In higher concentrations, the light is correspondingly stronger, plenty strong enough in fact, to be used for illuminating purposes could a sufficient quantity of the material be synthesized.

As Dubois (6) first demonstrated for *Pyrophorus* in 1885, so also in *Cypridina*, we may distinguish and separate two substances, in Dubois' terminology, luciferin and luciferase, which must be brought together before light will result. Contrary to my stand in previous papers of this series (7), I am now certain that Dubois' explanation of the action of luciferase and luciferin is quite incorrect, unless indeed light production be a different process in *Pholas dactylus* from that in *Cypridina* and the fire-fly. It is better perhaps to enter into a discussion of the rôle of luciferin and luciferase after the mechanism of light production and some of the properties of these two substances have been described.

The success of my investigations upon *Cypridina* and other luminous organisms was largely due to the interest and kindness of Prof. C. Ishikawa, of the Zoölogy Department, Agricultural College, Imperial University of Tokio, and to Prof. I. Ijima, of the Imperial University, who extended to me the hospitality of the Marine Biological Laboratory at Misaki. I am also indebted to Dean Kozai and Professor Aso of the Agricultural College for the use of many chemicals and apparatus. I extend my cordial thanks to all of these men.

GENERAL CHARACTER OF LIGHT AND OF LUMINOUS GLAND

Cypridina hilgendorfii is a strongly negatively heliotropic ostracod best obtained at night from fish heads, on which the animals feed, lowered into the water. It may be caught the year round, but is most abundant during August and September in Sagami Bay, Japan. Another non-luminous species (*Cypridina x*) is often obtained from the fish heads together with *C. hilgendorfii*. It is positively heliotropic to lamp-light. *C. hilgendorfii* is so strongly negatively heliotropic that it is not readily caught on moonlight nights and avoids a shore with many electric lights.

The light-giving material of *Cypridina* is a secretion formed in a special gland of yellow spindle shaped cells, opening near the mouth.[1] In adult living *Cypridinas*, the gland cells form a cylinder about 0.64 mm. by 0.24 mm., having a volume of about 0.0003 cm.[3] The secretion is readily formed upon agitation of the animal and is easily visible in a room in the daytime. To the dark adapted eye, the color is decidedly bluish and the light very intense. To a partially light adapted eye, the color is blue. In this respect the light differs markedly from the yellowish white (to the dark adapted eye) light of luminous bacteria, of *Cavernularia*, or of the Japanese fire-fly, which is green to the partially dark adapted eye. There is no inhibition of the light secretion in daylight as has been described for other forms (8). When first caught, *Cypridinas* give off the secretion readily, sometimes upon mere contact with the surface film of water, but if they are kept in the laboratory the secretion is not given off so readily. Removal from sea water also inhibits the ejection of the secretion and it is necessary to squeeze the animal rather strongly before the secretion is given out.

Electrical stimulation also calls forth the production of an abundant light secretion.

NATURE OF THE LIGHT SECRETION

The gland cells are almost completely filled with yellow material which can be observed to be composed of yellow globules 2 to 6 micra in diameter. These are extruded and dissolve to a colorless solution, absolutely free of visible granules, which gives the light. The globules are rather fluid in consistency as the outline can sometimes be seen to pass through amoeboid-like changes.

If we examine the natural secretion of *Cypridina* in sea water at night, the light appears perfectly homogeneous. No points of light appear such as might be due to granules, a condition wholly different from that in the juice of *Cavernularia* (4), which contains visible granules and at night the light from these under the microscope gives the appearance of a starry sky.

If *Cypridinas* be ground in a mortar and then examined at night, numerous very bright points of light appear and these are greatly increased by adding fresh water to the preparation. The light undoubtedly comes from solution of the above described yellow globules and when they have dissolved no more points of light but a steady homogeneous glow is to be observed. Electrical stimulation by strong interrupted induced shocks does not intensify the light from an extract of *Cypridinas*, glowing faintly.

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[1] For a description of the anatomy of the organ see Doflein, F., Sitzungsber. d. Ges. f. Morph. u. Physiol. in München., 1906, xxii, 133 and a paper by N. Yatsu in course of preparation.

PORCELAIN FILTRATION

Although no visible granules are found in the natural secretion of *Cypridina*, an ultramicroscopic investigation was not undertaken on account of lack of apparatus.

All of the light-producing substances (both photogenin and photophelein, p. 324) will easily pass a Pasteur-Chamberlain or a Berkefeld filter tube so that the particles present, if any, must be exceedingly small.

Complete proof of the truly soluble character of the light-producing substances is given by dialysis experiments recorded on page 329.

LACK OF OXYGEN

Oxygen is necessary for light production as may be seen by placing the crushed animals in a hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears even after a long time, but remains dim so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. Upon readmitting oxygen, however, a brilliant glow results. Every other species of animal investigated likewise requires oxygen for phosphorescence.

DESICCATION AND ETHER EXTRACTION

Like the fire-fly, the *Cypridinas* may be dried over $CaCl_2$ and will give a brilliant light if crushed and moistened with sea water. Dried crushed *Cypridinas* may be extracted with six changes of ether during the course of two days without impairing in the least their power to produce light when again moistened. The luminous substance is therefore of a non-lipoid, ether insoluble nature as might be expected from the fact that it is extruded from the animal as a clear water soluble non-fluorescent secretion. The luminous substance of the firefly and bacteria are also non-lipoid in nature (9).

CHEMICAL TESTS ON THE SECRETION

Despite the fact that the light from the natural secretion of *Cypridina* is very bright a sample of the secretion, collected by shaking many *Cypridinas* in a small volume of sea water and filtering, responds to none of the common biochemical tests. It gives no precipitate with picric acid or on saturation with $(NH_4)_2SO_4$ or on boiling, even when made slightly acid. Fehling's reaction is negative as also the biuret and xanthroproteic for proteins, the Molish reaction for carbohydrates and the indophenol test (a-napthol and para-phenylen-diamine) + H_2O_2 for oxidases.

I do not mean to infer from this that the luminous substance is neither protein, fat, nor carbohydrate, but merely that the concentration giving a bright light is too small to respond to chemical tests (see p. 336).

LUCIFERIN AND LUCIFERASE

The light from the natural secretion of *Cypridina* lasts for some time in sea water and then disappears and no amount of shaking will cause it to appear again. If we add to this natural secretion an extract of *Cypridina* heated to boiling, a brilliant light again results; or if we mix a water extract of *Cypridina* whose light has disappeared on standing with a similar extract whose light has been destroyed by boiling, light again results. In Dubois' phraseology we have mixed two substances, luciferin (in the boiled tube) and luciferase (in the tube allowed to stand) necessary for light production. According to Dubois, one of these, luciferase, is an oxidizing enzyme and is destroyed by heat; the other, luciferin, a substance not destroyed by heat is capable of oxidation with light production by means of luciferase. When the natural secretion of *Cypridina* is allowed to stand all the luciferin is oxidized and the luciferase is left; when a luminous extract of *Cypridina* is boiled the luciferase is destroyed before all the luciferin is oxidized.

At one time I believed Dubois' interpretation of this experiment to be correct, but results on *Cypridina* have led me to wholly different conclusions regarding the existence of luciferin and luciferase. Dubois' interpretation is indeed attractive. We know that the light production is an oxidation, that two substances are concerned, that these substances give light in very small concentration comparable with enzyme activity (see p. 336), that one of them can use up a large amount of the other (see p. 337) and possesses certain properties (destruction by heat, phosphotungstic and tannic acid) characteristic of enzymes. Further, we actually know of oxidative reactions taking place with the production of light under the action of oxidizing enzymes from plants and animals (10).

It is quite possible that light production in *Pholas dactylus* is of this nature as it differs radically in very essential points from the mechanism in *Cypridina* and in the fire-fly. Thus, Dubois finds that *Pholas luciferin* (the substance not readily destroyed by heat and giving light with luciferase) can be oxidized with light production by many oxidizing agents—blood, H_2O_2, $KMnO_4$, BaO_2, PbO_2, etc.—and that it occurs only in the luminous organs of *Pholas dactylus* (5). I find that *Cypridina* luciferin (a substance not readily destroyed by heat and giving light with luciferase) will not give light with the above mentioned oxidizing agents and is found in many other non-luminous animals and in the non-luminous parts of *Cypridina hilgendorfii.*

Dubois finds that *Pholas* luciferase (a substance destroyed easily by heat and using up *Pholas* luciferin with light production) occurs in many other non-luminous animals (5) whereas I find a body with the same properties only in the luminous organs of *Cypridina.* This substance, which we may temporarily call *Cypridina* luciferase, in concentrated solutions, will give light (as before mentioned) with extracts of many non-luminous animals (see p. 326). It will also give light if mixed with many pure substances as chloroform, ether, benzol, thymol, saponin, oleic acid, atropin, NaCl and others. Since most of the above substances could not possibly be oxidized by the luciferase, I conclude that they cause in some way the giving out of light in what Dubois terms luciferase. On this view luciferase is the source of the light and the luciferin (in the boiled extract of *Cypridina*) is something which causes the luciferase to emit light.

The substances causing the emission of light from a concentrated solution of luciferase are similar to those producing cytolysis of cells and I have considered the possibility that the concentrated extract might contain fragments of the luminous gland cells which cytolyse with light production or possibly granules which dissolve with light production as described in many other forms and as especially prominent in the juice of *Cavernularia.* I am, however, convinced that there are in the extract of *Cypridinas* which will give light with unoxidizable substances no cell fragments present and no granules above those of submicroscopic colloidal dimensions, for the following reasons:

First, the light is always perfectly homogeneous and in marked contrast to the points of light of *Cavernularia* juice where visible granules and cell fragments do occur.

Second, *Cypridina* extract (luciferase) will give light with thymol, butyl alcohol, or NaCl crystals after filtration through a Chamberlain or a Berkefeld filter, which removes all cell fragments.

PHOTOPHELEIN AND PHOTOGENIN

I conclude, therefore, that Dubois' luciferase, a thermolabile substance in luminous cells, is the light producing body. It gives light in contact with many substances not necessarily oxidizable and an especially bright light with a thermostabile (Dubois' luciferin) substance found in high concentration throughout the body of *Cypridina hilgendorfii* and in small concentration in the blood or juices of other non-luminous invertebrates. I therefore propose the new names photogenin (light producer from phos, light and gennao, to produce) for luciferase and photophelein (light assistor from phos, light and opheleo, to assist) for luciferin.

As we shall see later (p. 337) the photophelein can not be regarded as an enzyme because it is used up in the reaction and will not give light with an indefinite amount of photogenin. The photogenin corresponds much more to an enzyme but it is also slowly used up (p. 337). At present we can only speak of photophelein as a definite substance causing in some way the emission of light from photogenin.

Perhaps the following comparison of the important facts concerning light production in *Pholas* and *Cypridina* will make clearer Dubois' and my own views, and bring out the contradictory properties of the corresponding substances in the two animals.

	PHOLAS		CYPRIDINA	
	Luciferin	Luciferase	Photophelein	Photogenin
	Oxidises with light production by luciferase	Enzyme accelerating oxidation of luciferine	Substance assisting in production of light by photogenin	Auto-oxidisable in presence of photophelein
Destruction by heat	−	+	−	+
Dialysable	−	+	−	+
Stability	+	−	−	+
Occurrence in non-luminous parts	−	+	+	−
Oxidized by $KMnO_4$,H_2O_2 etc	+	−	−	−

Whatever the exact interpretation of the facts may be, it is certain that two substances are concerned in light production and these may be separated because they are destroyed at different temperatures. We may now inquire into each of their properties separately and return to a discussion of the mechanism of light production, in considering the possible enzyme nature of photogenin.

DISTRIBUTION OF PHOTOPHELEIN AND PHOTOGENIN IN ORGANISMS

Certain fundamental facts for the chemical theory of light production appear when we study the distribution of photophelein and photogenin —(I) in the non-luminous parts of *Cypridina;* (II) in non-luminous *Cypridinas* (*Cypridina x*); (III) in other non-luminous organisms; (IV) in luminous organisms.

By a careful quick scissors cut, the head end of *Cypridina* containing the luminous gland can be separated from the posterior half without any contamination of the latter with luminous secretion. If we now test the non-luminous half with dilute photophelein and photogenin, we find that it contains nothing which will give light with photophelein but something which will give a bright light with photogenin. We must try the experiment immediately because this substance disappears if the extract stands in presence of oxygen. In absence of oxygen or if the extract is boiled immediately (but not too long a time) the substance does not completely disappear even after one hour. There is, therefore, in the non-luminous parts, the substance photophelein which disappears even in the absence of photogenin (from luminous gland) unless the solution be boiled or oxygen excluded. Note the similarity to the disappearance of photophelein in presence of photogenin with this exception, that no light is produced. The experiment seems to indicate a third substance destroyed by heat, in non-luminous parts, which oxidizes (since oxygen is necessary) the photophelein.

In the extract of the non-luminous species, there is also a similar substance (photophelein) which will give light with photogenin. Unlike the photophelein from the non-luminous part of *Cypridina hilgendorfii,* it occurs in very small concentration, so that we must use concentrated[2] photogenin and concentrated extract of *Cypridina x.*

The photophelein from *Cypridina x* is also destroyed or disappears if the extract stands one and one-half hours in contact with oxygen but not in the absence of oxygen. Boiling makes the extract more stable.

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[2] By concentrated photogenin is meant an extract of one *Cypridina* in 0.5 to 1.0 cc. water.

In some other non-luminous forms widely different in relationship from *Cypridina*, there are substances which give light with concentrated photogenin and others which will not, whether the extract has been boiled or left unboiled. The extracts were boiled in order to destroy substances which in turn might quickly destroy photophelein. Among the light-giving extracts may be mentioned the following:

ANIMAL	ORDER	CHARACTER OF LIGHT FROM JUICE	
		Unboiled	Boiled
Sepia esculenta (blood)	Cephalopoda	Fair	Fair
Dolabella sp. (blood)	Gasteropoda	Faint	Fair
Laonome japonica (blood)	Annelida	Very faint	Very faint
Panulirus japonica (blood)	Decapodal	Very faint	Faint
Lepas anatifera (extract)	Cirripedia	Good	Bright
Tetraclita porosa (extract)	Cirripedia	Faint	Fair
Mitella mitella (extract)	Cirripedia	Faint	Fair
Oxyrhynchidae (moss crab) (extract)	Decapoda	Faint	Fair
Palaemon sp. (extract)	Decapoda	Faint	Faint
Chiton (extract)	Amphineura	Fair	Good
Harmithoe imbricata (extract)	Annelida	Fair	Fair
Cirratulus dasylophyus (extract)	Annelida	Very faint	Very faint
Marphysa iwamusi (extract)	Annelida	Faint	Faint
Onchidium sp. (extract)	Gasteropoda	Faint	Faint
Non-luminous parts fire-fly (extract)	Insecta	Negative	Fair
Flat worm (sp?) (extract)	Turbellaria	Faint	Faint

Of these forms *Lepas* and *Chiton* gave the best light and of these two only *Lepas* gave light with dilute *Cypridina* photogenin. Too much stress must not be laid upon comparative results because much depends upon the concentration and it is not easy to obtain extracts of comparative concentration.

Unlike the photophelein from the luminous parts of *Cypridina* or from non-luminous *Cypridinas*, that of *Lepas* extract or of *Dolabella* blood is perfectly stabile and will give light with photogenin even after standing a period of 24 hours.

Many extracts were found to give no light with concentrated photogenin. These included the following, which were tried both boiled and unboiled. Again it is possible that with greater concentrations even these extracts would call forth a faint light.

Animal	*Order*
Modiolus sp. ?	Lamellibranch
Acmaea sp. ?	Gasteropod
Sistrum sp. ?	Gasteropod
Sphaerechinus pulcherrinus	Echinoid
Wall crab (sp. ?)	Decapod
Ligia exotica	Isopod
Coccinella 7-punctata	Coleoptera
Anomala rufscuprea	Coleoptera
Glysiphana jocunda	Coleoptera

The following fluids and dissolved protein substances also give no light when their solutions were mixed with photogenin.

50 per cent egg albumen
50 per cent egg yolk
Na nucleate
Na nucleoproteinate
Witte's peptone
Neutral meat peptone
Dried ox blood extract

It is certain, then, that there is photophelein or something similar to it in the blood or extracts of many invertebrates but not necessarily in solutions of protein substances such as egg albumen, peptone, etc. In saliva there is something giving a very faint light and something in urine giving a fairly bright light with photogenin. Certain fluids tried were sufficiently acid to prevent the appearance of light and some fortuitous characteristic such as acidity may explain why extracts of some invertebrates give no light with photogenin.

It is hardly worth inquiring into the nature of the substances in each particular extract which may for convenience be collectively spoken of as photophelein since I have found a great many simple bodies, which, mixed with concentrated photogenin in powder or crystal form, give rise to a bright light. With more dilute solutions (one *Cypridina* to 10 cc.) no light appears. These substances include thymol, chloroform, hydrochinon, NaCl, butyl alcohol, saponin, oleic acid, ether, benzol, atropin, pilocarpin and ortol. Chloral hydrate, pyrocatechin, $MgSO_4$, $(NH_4)_2SO_4$, aesculin, dextrine and $K_4Fe(CN)_6$ gave a fainter light while saccharose gave none.

It is difficult to see in just what way all these diverse substances act. One is inclined to compare the production of light by chloroform or saponin to a process of cytolysis or to a stimulus reaction—like artificial parthenogenesis. The photophelein of *Cypridina* or other extracts would then be comparable to the cytolytic substance in the blood of worms which causes development of sea urchin eggs (11). The chloroform or saponin might be supposed to cause the solution of granules or globules (as can be observed in many cells) and the production of light to be connected with this solution process. But I have already given evidence (p. 324) to show that there are no granules unless ultramicroscopic ones in the light secretion of *Cypridina*, so that we must look to another interpretation.

Of many animal extracts tried on both concentrated and dilute photophelein, none will give light. Neither have I been able to obtain light with any chemicals or the oxidizing substances; neutral H_2O_2, BaO_2, ox blood alone or with neutral H_2O_2 added, neutral potato juice alone or with neutral H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, $FeCl_3$, or $K_4Fe(CN)_6$. As already pointed out (p. 323) this result is the direct opposite of the case in *Pholas dactylus* as described by Dubois.

There is, then, in the non-luminous parts of *Cypridina hilgendorfii* a large amount of photophelein but only a small amount even in a closely related non-luminous form. There appear to be only small amounts in other luminous forms not closely related to *Cypridina*. For instance, *Cypridina* photogenin will give a faint light with fire-fly photophelein but it is not nearly so bright as with *Cypridina* photophelein nor as bright as that given by non-luminous *Lepas* extract. Unfortunately, no closely related luminous crustacean was available so that the degree of specificity of photogenin and photophelein could not be determined. At least we may say that *Cypridina* photogenin and photophelein are specific to a certain extent, but not in the strict sense of the word.

The following tabulations give the luminous animals which have been tried with *Cypridina* in concentrated solution and the character of the light resulting. None gives light in more dilute solution.

Cypridina photophelein × Fire-fly photogenin	faint
Fire-fly photophelein × Cypridina photogenin	faint
Fire-fly photophelein × Fire-fly photogenin	bright

Cypridina[3] photophelein × Cavernularia photogenin	fair
Cavernularia photophelein × Cypridina photogenin	negative
Cavernularia photophelein × Cavernularia photogenin	negative

Cypridina photophelein × Noctiluca[4] photogenin	faint
Noctiluca[4] photophelein × Cypridina photogenin	faint
Noctiluca[4] photophelein × Noctiluca[4] photogenin	negative

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[3] An extract prepared with sea water, as the dark juice of *Cavernularia* gives a brilliant light with fresh water.

[4] All the *Noctiluca* extracts were made with n/1000 NH_4OH to neutralize the acid of the *Noctiluca* vacuoles.

Owing to lack of material, the luminous fish, *Monocentris japonica* could only be tested in more dilute solutions, and with negative results, viz.:

Cypridina photophelein × Monocentris photogenin............negative
Monocentris photophelein × Cypridina photogenin............negative
Monocentris photophelein × Monocentris photogenin..........negative

DIALYSIS

Photophelein will dialyze through heavy parchment and collodion fairly readily, in the case of collodion sometimes appearing in the dialysate in the course of two hours.

Photogenin dialyzes with difficulty and with some collodion tubes not after a period of thirty-six hours. In others a very slight amount will pass in that time and in one experiment with heavy parchment paper a very slight dialysis occurred in twelve hours but usually there was none. The collodion tubes and the parchment paper did not leak in any of the experiments recorded.

ADSORPTION

Both photophelein and photogenin are removed from solution by washed boneblack and washed freshly precipitated $Fe(OH)_3$. To serve as control the last washings from boneblack and $Fe(OH)_3$ were added to photogenin and photophelein and tested with photophelein and photogenin respectively to make sure that the adsorption was not apparent and due to destruction by foreign substances from the adsorbing media.

TEMPERATURE

The exact temperature of destruction of photophelein and photogenin depends upon their concentration and the time of heating. *Cypridinas* dried over $CaCl_2$, ground, and the powder suspended in sea water give a beautiful light which disappears when heated to 56°, but returns on cooling. If heated to 65° and cooled, the light also returns, but does not return if heated to 70° and then cooled. A very concentrated mixture of photogenin and photophelein may be heated above 70° and whole *Cypridinas* heated to boiling will occasionally give a faint light when cooled. The light from the normal secretion of *Cypridina* disappears at 52°–54°, and returns on cooling, so that we may regard this as the inhibition temperature and something above 70°, depending on the concentration, as the destruction temperature.

Other organisms give the following inhibition or destruction temperatures:[5]

Luminous bacteria (17) 38°	Recovery if cooled immediately
Hydroid, Sertularia 54°	No recovery on cooling
Noctiluca (2) 48°	No recovery on cooling
Cavernularia (4) 52°	No recovery on cooling
Luciola (3) 42°	No recovery on cooling
Pholas dactylus (5) 60°	Recovery?

The time necessary to destroy photophelein at 100° depends also upon its concentration. In dilute solution (one *Cypridina* to 25 or 50 cc. water) boiling for one minute is sufficient but in concentrated solution (one *Cypridina* to 1 cc. water) five minutes boiling is necessary.

In *Pholas dactylus* (5) photophelein (luciferin) is destroyed above 70° whereas in the fire-fly (both *Photuris* and *Luciola*) it may be boiled for ten minutes without destruction.

The luminous material of *Cypridina* like that of most luminous organisms is unaffected by cold and will glow brilliantly at 0°C.

STABILITY

Cypridina photogenin is much more stabile than photophelein. The time after preparation that one can obtain light from these substances depends largely upon their concentration as also upon the temperature. One sample of concentrated (one *Cypridina* to 2 cc.) photogenin gave light with fresh photophelein for seven days at 20° despite the fact that decomposition had taken place and the liquid smelt foul. Dilute photogenin (one *Cypridina* to 50 cc.) may lose its power in one day; very dilute photogenin (one *Cypridina* to 12,500 cc.) in less than two hours.

Cypridina photophelein also disappears from solution spontaneously, and the more dilute the solution the more quickly does it lose its power to phosphoresce. With one *Cypridina* to 25 cc. the power disappears in three to four hours, while with one *Cypridina* to 1 cc. it may last for five days at 26° to 28°C.

The decomposition occurs whether the solution be neutral, acid (n/8000 HCl) or alkaline (n/4000 NaOH), in pure water or sea water, in the light and also in amber bottles, and occurs even if the photophelein be thoroughly boiled to destroy all traces of photogenin which might slowly use up the photophelein without light production (see p. 337).

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[5] See also the table in Mangold's article, Die Production von Licht, in Winterstein's Vergleichende Physiologie, Vol. 3, part 2, 345.

The photophelein is apparently oxidized, as the destruction is not so rapid in absence of oxygen.

PRESERVATIVES AND ANESTHETICS

The addition of preservatives (anesthetics) as chloroform, ether, benzol and thymol is to hasten the spontaneous destruction of the photophelein and to preserve the photogenin. Ether is especially destructive to the photophelein whereas photogenin will give light after saturation with ether for twenty-two days. The following tables (1 and 2) show the effect of saturation of solutions (one *Cypridina* to 25 cc.) of photophelein and photogenin with the four substances.

TABLE 1

Effect of preservatives on photophelein

SATURATION WITH	TESTED WITH PHOTOGENIN AFTER			
	5 minutes	1 hour	1 day	2 days
Ether	Bright light	Negative	Negative	Negative
Benzol,	Bright light	Very, very faint	Negative	Negative
Chloroform	Bright light	Very faint	Negative	Negative
Thymol	Bright light	Faint	Negative	Negative
Control	Bright light	Bright light	Faint[6]	Negative

TABLE 2

Effect of preservatives on photogenin

SATURATION WITH	TESTED WITH PHOTOPHELEIN AFTER					
	1 day	2 days	7 days	22 days	56 days	130 days
Ether	Bright	Bright	Faint	Very faint	Negative	Negative
Benzol	Bright	Bright	Bright	Negative		
Chloroform	Bright	Bright	Bright	Bright	Bright	
Thymol	Bright	Bright	Faint	Faint	Negative	
Control	Bright	Faint	Negative			

The harmlessness of the above anesthetics for *Cypridina* photogenin is unusual as Dubois found a marked destructive action on *Pholas* photogenin (luciferase) and I have noted the same thing for the fire-fly.

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[6] Often photophelein will not last for one day.

As we have just seen, the addition of certain anesthetics does not rapidly destroy photophelein or photogenin. We can saturate a phosphorescent mixture of the two with ether and the light will still last for some time. If we add butyl alcohol to saturation the light disappears and if the solution is now diluted with water or sea water, *the light reappears*. The same phenomenon is observed if the photogenin be filtered through a Chamberlain porcelain filter to remove all traces of cells or cell fragments. Care was taken to make sure that the return of light was not due to fluid adherent to the sides of the test tube and untouched by the butyl alcohol.

A similar phenomenon is observed with ethyl alcohol and acetone. If we add in small amounts absolute ethyl alcohol to a glowing mixture of photogenin and photophelein, the light becomes very dim when 16 per cent alcohol has been added and disappears with 20 per cent alcohol. If now the mixture be diluted the light returns. Acetone[7] behaves as alcohol. About 23 per cent is necessary for extinction of the light. Saturation with chloretone does not extinguish the light.

The effect of ethyl alcohol and acetone might be explained as the effect of precipitation, because of insolubility in the 20 per cent solution but we can not so explain the extinction of the light by butyl alcohol and subsequent recovery on dilution since butyl alcohol is only soluble to the extent of 8.3 parts in 100 parts of water. We are dealing with a highly interesting effect—one akin to anesthesia—a reversible inhibition, not of a cell or cell fragments but of a solution. Filtration through porous porcelain shows that no cell fragments can be present. I can not here enter more fully into a discussion of this interesting phenomenon, whose bearing on anesthesia is obvious, except to point out that if we can anesthetize a solution, we need not, as some recent theories have done, regard changes in the cell membrane to be necessarily the ultimate cause of anesthesia.

It may be pointed out in passing that the production of light gives us the opportunity of observing the effect of, let us say, an anesthetic or temperature, upon a process at any particular instant, rarely obtained in the study of enzyme action or even of cell action except where some movement is affected. For instance, we can heat pepsin to 60° and then cool it and see if it will digest protein. If we find that it will digest protein, that gives us no information as to a reversible inhibition at 60°, an inhibition which might become non-reversible if we kept the pepsin at 60° long enough to test directly the power of digestion. We can, however, heat photogenin and photophelein to 60°, cool them and find out immediately if they give light.

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[7] The sample of acetone at hand was not particularly pure.

PROTEIN PRECIPITANTS

The light disappears in a phosphorescent mixture of photogenin and photophelein when the following substances are added to the concentrations indicated: tannin—m/2500 to m/5000; phosphotungstic acid—m/2500 to m/5000; picric acid—m/500, assuming a saturated water solution to be 1.03 per cent or m/22.

A peculiar phenomenon observed with phosphotungstic and tannic acids but not with picric acid is, that, at the lower limit of extinction, the light reappears after first disappearing and more solution must be added in order to extinguish it again. Care was taken to make sure that the phenomenon was not due to more perfect adaptation of the eyes to the dark. When the light is extinguished by dilute picric acid it will reappear again on dilution with water.

ACIDS AND ALKALIES

The effect of acids (HCl) and alkalies (NaOH) can be seen by adding 1 cc. of the acid (or alkali) of a definite concentration to 1 cc. of a glowing mixture of photogenin and photophelein. The concentration of the acid or alkali in the mixture will thus be one-half of the concentration added. The results are given in table 3.

TABLE 3

Effect of acids and alkalies on light production

CONCENTRATION IN MIXTURE	CHARACTER OF LIGHT
n/250 NaOH	Instantly disappears
n/500 NaOH	Instantly very dim, lasting > 5 minutes
n/1000 NaOH	Instantly dim, lasting > 5 minutes
n/2000 NaOH	Instantly fair, lasting > 5 minutes
n/4000 NaOH	Instantly fair, lasting > 5 minutes
n/8000 NaOH	Instantly fair, lasting > 5 minutes
Control	Remains good > 5 minutes
n/2000 HCl	Instantly disappears
n/4000 HCl	Instantly dim, lasting > 5 minutes
n/8000 HCl	Remains good > 5 minutes

The effect of acid (HCl) and alkali (NaOH) was also studied in the following way: 1 cc. fairly dilute (one *Cypridina* to 10 cc.) photophelein was mixed with 1 cc. n/500 HCl, allowed to stand one hour and then neutralized by adding an equal volume of n/1000 NaOH and tested for light by adding photogenin. The photophelein was therefore in contact with n/1000 HCl for one hour. In this way the effect of acid on photogenin and photophelein can be separated. The results are given in table 4.

TABLE 4

Effect of acid and alkali on photophelein and photogenin

CONCENTRATION OF HCl	EFFECT ON PHOTOPHELEIN NEUTRALIZED AFTER ONE HOUR AND PHOTOGENIN ADDED	EFFECT ON PHOTOGENIN NEUTRALIZED AFTER ONE HOUR AND PHOTOPHELEIN ADDED
n/1000	Negative	Negative
n/2000	Very faint	Negative
n/4000	Bright	Very faint
n/8000	Bright	Bright
Control in water	Bright	Bright
CONCENTRATION OF NaOH		
n/31	Faint	Negative
n/62	Faint	Negative
n/125	Faint	Negative
n/250	Faint	Faint
n/500	Faint	Fair
n/1000	Faint	Fair
Control in water	Good faint	Fair

Even very small concentrations of NaOH added to a glowing mixture of photogenin and photophelein will decrease greatly the amount of light (table 3). Thus if we add 1 cc. n/500 NaOH to 1 cc. of a brightly glowing mixture, making the concentration of NaOH n/1000, the light instantly becomes very dim and remains so for a considerable time. If NaOH be added to a concentration of n/250, the light disappears completely; if we now neutralize the NaOH with n/250 HCl, the light returns, faint.

Still smaller concentrations of HCl prevent light production. Thus, (table 3) n/2000 HCl extinguishes the light from a luminous mixture, but on neutralization with n/2000 NaOH, the light returns and is fairly bright. The effect of NaOH and HCl is therefore to inhibit light production, and not to immediately destroy the photogenin and photophelein (tables 3 and 4). Note also from table 4 that the photogenin is more readily affected by HCl and especially by NaOH than the photophelein.

In the case of acid and alkali as with so many other substances on the light-giving material, the effect is reversible if the substance is removed. The concentration of HCl which inhibits light production is the same as that affecting cells, although the concentration of NaOH necessary to inhibit light production is somewhat greater than that necessary to affect cells (15).

POTASSIUM CYANIDE

The effect of KCN is of especial interest because of its power of inhibiting cell oxidations. It was tested by mixing an equal volume of photogenin (one *Cypridina* to 25 cc.) with the KCN solution, and testing with an equal volume of photophelein (one *Cypridina* to 12 cc.) after ten minutes and after one hour. Table 5 gives the results.

TABLE 5

Effect of KCN on light production

CONCENTRATION OF KCN IN PHOTOGENIN SOLUTION	LIGHT WITH PHOTOPHELEIN AFTER 10 MINUTES	LIGHT WITH PHOTOPHELEIN AFTER 1 HOUR
n/20	Fair	
n/30	Fair	Fair
n/125	Fair	Fair
n/250	Bright	Bright

In *Cypridina* as in all other luminous animals which I have tried (*Cavernularia* (4), *Noctiluca* (2), fire-fly (3), luminous bacteria),[8] KCN is practically without influence on light production. *Cavernularia* juice, for instance, will light for over 90 minutes in n/40 KCN. On the other hand, n/1280 KCN is sufficient to completely inhibit the oxyluminescence of pyrogallol by the vegetable oxidases (10).

SATURATION WITH SUGAR, $(NH_4)_2SO_4$ AND NaCl

Saturation of a luminous mixture of photogenin and photophelein with sugar or NaCl causes the light to disappear and it reappears on dilution of the mixture with water.

Since $(NH_4)_2SO_4$ is acid a small amount of this salt causes the light to disappear and it does not return upon dilution.

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[8] Unpublished experiments.

Probably these phenomena are connected with the salting out of the luminous substances, although no signs of a precipitate are visible when the natural secretion of *Cypridina* is saturated with NaCl or $(NH_4)_2SO_4$. As already pointed out, this result is no doubt due to the small concentrations of the substances present.

CONCENTRATION OF PHOTOGENIN AND PHOTOPHELEIN IN *Cypridina*

In the normal secretion of *Cypridina*, there is more photogenin than photophelein, as may be seen by adding fresh photophelein to the normal secretion after the light has disappeared on standing. The light again appears. The photophelein had been completely used up. This may be shown in another way by allowing a concentrated mixture of photogenin and photophelein to stand until the light disappears, and then boiling one half of the mixture. Upon mixing the two halves no light results, as all the photophelein had been used up before the photogenin was destroyed in the tube boiled.

The concentrations of the two substances which will give visible light when mixed are very small. In one experiment performed 15 minutes[9] after the photophelein was obtained, one *Cypridina* in 25,600 cc. of water gave a just visible light when mixed with an equal volume of more concentrated photogenin. The photogenin from one *Cypridina* in 25,600 cc. water will also just give visible light when mixed with an equal volume of photophelein.

We can see how small an amount of photogenin will give light when we consider that the animal is about 3.5 by 2.5 by 1.5 mm. and that the luminous gland is more or less of a cylinder 0.64 high by 0.24 mm. diameter with a volume of about 0.03 mm. or 0.00003 cm. and that one luminous gland in 25,600 cc. water will give visible light when mixed with an equal volume of photophelein, or in a concentration of 51,200 cc. Hence one part of gland in about 1,700,000,000 parts of water will give visible light upon the addition of photophelein. Even this is a low estimate, as we do not know what the concentration of the photogenin is in the gland itself.

When we compare the amount of photogenin which can be detected by light production with the amount of substances detectible by chemical means we see how extraordinarily sensitive the light reaction is. AgCl is soluble in water to the extent of one part in 625,000 parts water at 20° (12) so that more chloride than this must be present to be detected by $AgNO_3$, a very delicate reaction. $KMnO_4$ is roughly just distinguishable by its color in a 14 mm. test tube in a concentration of 1: 1,250,000.

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[9] The experiment was performed as quickly as possible because the photophelein disappears on standing.

Biological reactions are, generally speaking, more delicate than inorganic reactions. Zinc in traces has a favorable influence on the growth of moulds and 1: 25,000,000 will increase the weight of a crop of *Aspergillus* by 50 per cent (13). Copper will kill *Spirogyra* in one minute when present in 1: 77,000,000 concentration (13). According to Kastle (14), blood in 1: 80,000,000 can be recognized by the phenolphthalin reaction. Since this reaction is due to hemoglobin, of which blood contains about 10 per cent, the hemoglobin can be recognized in 1: 800,000,000 concentration. These figures give an idea of the delicacy of the photogenin-photophelein reaction as compared with others. It should be borne in mind that pyrogallol in 1: 254,000 will give visible light with potato juice and H_2O_2 (10).

IS PHOTOGENIN USED UP IN LIGHT PRODUCTION WITH PHOTOPHELEIN?

The exceedingly small concentration of light substances which give visible light suggests that one or both are of enzyme nature as Dubois supposes. There are two ways of testing this question. One is to determine whether a small concentration of photogenin can use up a large concentration of photophelein providing a sufficiently long time is allowed. We can not use this method because photophelein decomposes spontaneously.

Another way is to determine if a small amount of photogenin can decompose successively added amounts of photophelein without itself undergoing diminution. This method is not unequivocal since many true enzymes are paralyzed or destroyed by the decomposition products of the reaction which they accelerate.

It was found that if we add to 1 cc. of a weak (one *Cypridina* to 50 cc.) solution of photogenin, successive 1 cc. additions of a concentrated (one *Cypridina* to 2 cc.) solution of photophelein as soon as the light from the preceding addition has disappeared, after four 1 cc. additions no more light will appear. The photogenin is therefore used up and can not oxidize additional photophelein, although there is plenty of photophelein present, as can be shown by adding fresh photogenin when a good light appears. With each successive addition of concentrated photophelein, the light which at first is very bright and lasts about one hour, becomes less brilliant and lasts a shorter time. This is not due to mere dilution of the dilute photogenin as we can dilute the dilute photogenin to the same volume with water and then upon adding photophelein a good light results. We can only conclude that, although photogenin can use up a large amount of photophelein, it is itself changed in some way in the reaction and disappears. We can not say how much photophelein will combine with a definite quantity of photogenin because we do not know the absolute amount of these substances in a single *Cypridina*. In the above experiment we added a concentration of photophelein from one *Cypridina* 100 times (i.e., four additions each 25 times more concentrated), that of the photogenin from one *Cypridina*.

Although the evidence goes to show that the photogenin is used up it is not nearly so rapidly used up as is the oxidase of potato in the production of light from pyrogallol (10). We must remember also that certain enzymes, as zymase, (thermolabile and non-dialyzable) are only active in presence of a co-enzyme which is not destroyed by heat and is easily dialyzable. In fact the photogenin-photophelein system resembles to a very remarkable degree the zymase-co-zymase system. There is the same quantitative relation between zymase and co-zymase as between photogenin and photophelein. If zymase is present in excess, the co-enzyme is all used up; if co-zymase is in excess then the zymase is used up (16). Nevertheless, however much photogenin resembles co-zymase, I have for the present deemed it best to avoid the termination ase. In absence of more definite knowledge we may provisionally regard photogenin as a substance autoöxidizable only in presence of photophelein.

If we try the reverse experiment, that of adding 1 cc. of concentrated (one *Cypridina* to 2 cc.) photogenin to 1 cc. of dilute (one *Cypridina* to 50 cc.) photophelein, a bright light lasting ten to fifteen seconds appears and no more light upon adding additional concentrated photogenin. As might be expected the small amount of photophelein is very rapidly used up by the large amount of photogenin.

The quantitative relations between concentration of photophelein and photogenin, duration of light, and brightness of light, will be considered in a subsequent paper.

SUMMARY

1. The luminous secretion of *Cypridina* comes from several spindle shaped yellow gland cells on the upper lip and is extruded to the sea water as perfectly clear granule-free non-fluorescent secretion. The light is homogeneous and bluish white in color.

2. If the luminous secretion stands, the light disappears, and if we now add an extract of *Cypridinas* heated to boiling, the light again returns; i.e., *Cypridina* gives a luciferin-luciferase reaction similar to *Pholas dactylus* as described by Dubois.

3. Contrary to Dubois' theory, the luciferase is the source of the light, and not an enzyme causing light production by oxidation of luciferin, because we can obtain light from luciferase by substances incapable of oxidation (NaCl, chloroform, ether, etc.). The new names of photogenin or light producer for luciferase, and photophelein or light assistor for luciferin have therefore been proposed.

4. Oxygen is necessary for light production.

5. Both photogenin and photophelein will pass a Pasteur-Chamberlain or Berkefeld filter easily.

6. Photophelein dialyzes readily through heavy parchment or collodion, photogenin with great difficulty or not at all, even after a period of thirty-six hours.

7. Both photogenin and photophelein are adsorbed by bone-black and $Fe(OH)_3$.

8. The light-producing substances may be dried and thoroughly extracted with ether without impairing their light-giving power.

9. Chemical tests on the natural light secretion give negative results since a very small amount of light substance gives a bright light and at least one part of photogenin or photophelein in 1,700,000,000 parts of water will give a visible light.

10. Photophelein occurs throughout the body of *Cypridina*, photogenin only in the luminous organ. Photophelein from non-luminous parts disappears (apart from photogenin) on standing, but not so readily if the extract has been boiled or in the absence of oxygen. In the fire-fly and some non-luminous beetles, the photophelein disappears so quickly that it is best to make the extract with boiling water. In a non-luminous species of *Cypridina*, a photophelein occurs in small quantity with properties similar to that in *Cypridina hilgendorfii*.

11. Photophelein occurs in extracts (both boiled and unboiled) of many non-luminous organisms, in greatest quantity in *Lepas anati-*

fera. The photophelein of *Lepas anatifera* does not disappear on standing.

12. Pure protein solutions (peptone, Na nucleoproteinate, albumen, etc.) or dried mammalian blood do not contain photophelein, but urine contains a similar body.

13. While photogenin will give light with many substances known and unknown, photophelein will give light only with the photogenin of luminous organs.

14. *Cypridina* photogenin will give no brighter light with extracts (photophelein) of other luminous forms (*Luciola, Cavernularia, Noctiluca*) than with extracts of non-luminous forms, and *Luciola* photogenin will not give so bright a light with *Cypridina* photophelein as with boiled extracts of non-luminous insects. We must conclude that the two substances are not specific for luminous forms, although there is a certain amount of specificity, for photogenin gives the best light with photophelein from the same species.

15. Bright light can be formed by *Cypridina* at 0°C. Photogenin is destroyed above 70°, the temperature and time depending on the concentration; photophelein only after several minutes boiling, the time depending also on the concentration. The natural luminous secretion ceases to light at 52 to 54°, but the light returns on cooling.

16. Photophelein is relatively unstabile and disappears on standing, the time depending on the concentration. Photogenin is much more stabile but also disappears slowly.

17. The spontaneous decomposition of photophelein is retarded (perhaps prevented) by lack of oxygen but hastened by addition of preservatives (chloroform, ether, benzol, thymol). Photogenin can be kept longer by addition of preservatives. One sample preserved with chloroform retained its power to give light with photophelein for over fifty-six days at room temperature.

18. Saturation with ether, chloroform, benzol, thymol or chloretone does not affect the light from a mixture of photogenin and photophelein. Saturation with butyl alcohol or 20 per cent ethyl alcohol or 16 per cent acetone extinguishes the light, and if the mixture is diluted with water, the light reappears. This phenomenon of *anesthesia of a solution* is given by photogenin filtered through porcelain plus photophelein and difficultly soluble butyl alcohol, so that it can not be due to the presence of cell fragments or to insolubility in the 20 per cent ethyl alcohol.

19. Picric acid, tannic acid and phosphotungstic acid extinguish the light in very weak concentrations, and the light returns in the case of

picric acid if the solution is diluted with water. In the case of tannic and phosphotungstic acids, the light returns after first disappearing even *without diluting*.

20. HCl between n/2000 and n/4000 and NaOH between n/250 and n/500 extinguish the light, and the effect is reversible upon neutralization. It can be shown that both HCl and NaOH affect the photogenin more readily than the photophelein and that the effect of NaOH especially is more readily reversible.

21. KCN does not inhibit light production even in strong concentration.

22. Saturation with $(NH_4)_2SO_4$, NaCl or cane sugar extinguishes the light most readily in the order named, and the effect is reversible except in case of $(NH_4)_2SO_4$ (due to acidity).

23. In the natural secretion of *Cypridina* or in the whole animal there is always enough photogenin to completely use up the photophelein. The photogenin from one animal will use up a large additional amount (at least 100 times the concentration in one animal) of photophelein, but not an indefinite amount, so that photogenin is not a true enzyme in the strict sense of the word, unless it be an enzyme poisoned by its own reaction products. The photogenin-photophelein system resembles the zymase-co-zymase system to a remarkable degree, but it is best for the present to regard photogenin not as an enzyme but merely as a substance autoöxidizable only in presence of photophelein.

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STUDIES ON BIOLUMINESCENCE

V. THE CHEMISTRY OF LIGHT PRODUCTION BY THE FIRE-FLY

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Contribution from the Physiological Laboratory, Princeton University, and Department of Marine Biology, Carnegie Institution of Washington

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The experiments recorded herein have been made during a period of two years on several different species of fire-fly—the American *Photuris pennsylvanica* and *Photinus pyralis*, the Japanese *Luciola vitticollis* and *parva* and the West Indian "Cucullo," *Pyrophorus noctilucans* and *havaniensis* All the species are essentially similar in behavior, and in general the statements apply to all.

In the second paper of "Studies on Bioluminescence" (1) I pointed out that the fire-fly gave Dubois' (2) luciferin-luciferase reaction (i.e., an extract of the luminous organ allowed to stand until the light disappears will again give light if mixed with an extract of luminous gland which has been boiled), and that luciferin from one species or genus of fire-fly would give light with luciferase from another species and vice versa. In the fourth paper of the series, (3) I showed that in *Cypridina* Dubois' luciferase (the thermolabile substance) was the source of the light and not luciferin (the thermostabile substance) as Dubois' theory supposes, and I proposed that these substances be called photogenin (light producer), equivalent to luciferase, and photophelein (light assistor), equivalent to luciferin. Photogenin (luciferase) will use up a large amount of photophelein (luciferin) but itself also disappears in the process so that it seems best to avoid the enzyme termination *ase* for the present. Photogenin appears to be a substance auto-oxidizable with light production in presence of photophelein.

Since the fire-fly differs from *Pholas* and agrees with *Cypridina* in important points—in the fact that fire-fly photophelein (luciferin) can not be oxidized with oxidizing agents, and is found in many non-luminous forms—I have also applied the names photogenin and photophelein to the light producing substances of the fire-fly.

A brief account of the properties of fire-fly photogenin and photophelein as compared with other luminous forms follows.

DISTRIBUTION OF PHOTOGENIN AND PHOTOPHELEIN

While photogenin is found only in the luminous gland cells of the fire-fly, photophelein is distributed throughout the body of the fire-fly, the opposite of the condition described by Dubois for *Pholas*. An extract of the non-luminous parts of the fire-fly will give light with photogenin, but only if tested immediately the extract is made. An extract ten minutes old is found to contain no photophelein unless it has been previously boiled. After boiling the photophelein can be kept for many days without decomposition. There appears to be something in the extract of non-luminous parts of the fire-fly which causes the photophelein to disappear unless the former is destroyed by heat. That this is the case can be shown by adding unboiled extract of the non-luminous parts to photophelein, when the latter disappears from solution. Because of failure to boil the non-luminous fire-fly extract, I had previously overlooked the existence of photophelein in regions other than the luminous gland. Lack of oxygen retards (prevents?) the disappearance of photophelein from non-luminous parts, showing that an oxidation is involved. Note that just as photophelein disappears in contact with photogenin and oxygen with light production, so it also disappears in contact with this substance in non-luminous parts, but without light production. In this respect the fire-fly agrees perfectly with *Cypridina*.

Among non-luminous forms, we find some whose extract will give light with photogenin whether boiled or unboiled, others whose boiled extract only will give light, and others whose extract will give no light whether boiled or unboiled. The first mentioned presumably contain photophelein not readily destroyed by standing, the second are similar to the fire-fly and the third may contain no photophelein or some substance very quickly destructive to photophelein or an excess of acid or perhaps merely unstabile photophelein. The exact reasons have not yet been worked out. The following extracts were tried:

Luciola photogenin × caterpillar (sp?) blood..........................bright
Luciola photogenin × caterpillar (sp?) blood, boiled....................bright
Luciola photogenin × beetle (coccinella 7-punctata) extract...........negative
Luciola photogenin × beetle (coccinella 7-punctata) extract, boiled......bright
Luciola photogenin × beetle (Glysiphana jucunda) extract............negative
Luciola photogenin × beetle (Glysiphana jucunda) extract, boiled.......bright
Luciola photogenin × beetle (Anomala rufscuprea) extract............negative
Luciola photogenin × beetle (Anamola rufscuprea) extract, boiled.....negative
Luciola photogenin × grasshopper (Pachytylus danicus) extract.....very faint

Luciola photogenin × grasshopper (Pachytylus danicus) extract, boiled....faint
Luciola photogenin × myriapod (Scolopendra sp?) extract.................faint
Luciola photogenin × myriapod (Scolopendra sp?) extract, boiled.........faint
Luciola photogenin × ox blood extract (boiled or unboiled)............negative
Luciola photogenin × neutral potato juice (boiled or unboiled)........negative

Note that the oxidases of blood or potato juice will give no light with photogenin and they also will give no light with photophelein, even if we add H_2O_2. As pointed out in my previous papers, although the oxidases can oxidize pyrogallol with light production (4), they have nothing to do with light production by animals.

CONCENTRATION OF PHOTOGENIN AND PHOTOPHELEIN NECESSARY TO PRODUCE LIGHT

The amount of photogenin in the fire-fly is greater than the amount of photophelein necessary to combine with it; otherwise we would not be able to obtain photogenin because it would be completely used up in combination[1] with photophelein. In some luminous animals (*Noctiluca, Cavernularia, Watasenia*), I have utterly failed to demonstrate a photogenin-photophelein reaction under conditions which should be favorable and after many attempts to demonstrate it. The explanation of this result may lie in the presence of equivalent amounts of the two substances.

Both photogenin and photophelein are found in much smaller concentration in the fire-fly than in *Cypridina*. The photogenin from one fire-fly (*Luciola parva*) whose average size is 8.2 × 3.1 mm. with luminous organ in the ♂ 2.2 × 1.5 mm. and in the ♀ 2.3 × 0.5 mm., will give just visible light in 1.6 cc. water, and the photophelein from one fire-fly in 3.2 cc. water. The greater possible dilution of the photophelein is due no doubt to the fact that the photophelein comes from the whole body while the photogenin is derived only from the luminous gland. When we compare this with the crustacean, *Cypridina*, which will give visible light in 25,600 cc. or one part of luminous gland in 1,700,000,000 parts of water, we see that the fire-fly, despite the brilliancy of its light, is not a very powerful light producer.

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[1] I have not actually made the experiment to show that photogenin is used up in giving light with photophelein, but it seems highly probable that this is the case in the fire fly as it is in *Cypridina*.

Comparative properties of photogenin and photophelein in Cypridina, fire-fly and Pholas

PROPERTY	CYPRIDINA (HARVEY, 3)		FIRE-FLY		PHOLAS (DUBOIS, 2)	
	Photophelein	Photogenin	Photophelein	Photogenin	Luciferin (Photophelein)	Luciferase (Photogenin)
Destruction temperature	Boiling 1–5 minutes	70° or above. (light disappears 52–56° returns on cooling)	Not destroyed by 10 minutes boiling	42°	Above 70°	60°
Distribution	Throughout *Cypridina* and many non-luminous forms	Luminous organs of *Cypridina*	Throughout fire-fly and many non-luminous forms	Luminous organs of fire-fly	Luminous organ of *Pholas*	*Pholas* and many non-luminous forms
Stability	Less stabile	More stabile	More stabile	Less stabile	More stabile	Less stabile
Porcelain filtration	Positive	Positive	Positive		With difficulty	With difficulty
Dialysis	Easily	Very slowly	Easily	Negative (?)	Slow	Negative
Adsorption by bone-black and $Fe(OH)_3$	Positive	Positive	Negative			
Light with oxidizing agents	Negative	Negative	Negative	Negative	Positive	Negative
Light with saponin, thymol, NaCl, etc.	Negative	Positive	Negative	Negative		
Ether solubility	Negative	Negative	Negative	Negative	Negative	Negative
Concentration just giving light	1: 1,700,000,000	1: 1,700,000,000				
Substance from one animal gives just visible light in	25,600 cc.	25,600 cc.	3.2 cc. for *Luciola parva*	1.6 cc. for *Luciola parva*		

Comparative properties of photogenin and photophelein in Cypridina, fire-fly and Pholas—Continued

PROPERTY	CYPRIDINA (HARVEY, 3)		FIRE-FLY		PHOLAS (DUBOIS, 2)	
	Photophelein	Photogenin	Photophelein	Photogenin	Luciferin (Photophelein)	Luciferase (Photogenin)
Effect of chloroform, ether, benzol, etc.	Fairly stabile	Stabile	Stabile	Quickly affected	Very slowly affected	Slowly affected
Effect of alkalies	Not destroyed by n/30 NaOH in 1 hour	Destroyed by n/125 NaOH in 1 hour			Forms insoluble alkali-albumin with NH_4OH	
	Reversible extinction of light by n/125 NaOH if neutralized immediately					
Effect of acids	Destroyed by n/1000 HCl in 1 hour	Destroyed by n/2000 HCl in 1 hour			Not precipitated by acetic and H_2CO_3 except in presence of neutral salts	
	Reversible extinction of light by n/2000 HCl if neutralized immediately					
Effect of alcohols and acetone	Reversible extinction of light with 20 per cent alcohol, 16 per cent acetone and saturated butyl alcohol				Precipitated by alcohol at 82° unchanged	
Effect of tannic, picric and phosphotungstic acids	Reversible extinction of light in n/500–n/5000 concentration				Precipitated by picric acid unchanged	

Effect of KCN	No effect	No effect		
Effect of NaF	—	—		Not destroyed by 1:100
Effect of enzymes	—	—		Destroyed by trypsin
Saturation with $(NH_4)_2SO_4$, NaCl, $MgSO_4$, sugar and glycerine	Reversible extinction except in case of acid $(NH_4)_2SO_4$	—	Precipitated by $(NH_4)_2SO_4$, not by NaCl or $MgSO_4$, Insoluble glycerine	Suspend activity and returns on dilution
Color of light	Bluish white	Yellowish white	Greenish blue	

PROPERTIES OF PHOTOGENIN AND PHOTOPHELEIN

As to the chemical nature of photogenin and photophelein, nothing definite is known. The photophelein is much the more stable substance and can be preserved until attacked by bacteria or with chloroform, for over 70 days. It dialyses readily through collodion, is not adsorbed by lamp-black, and is not readily affected by ether and benzol. Photogenin, on the other hand, disappears in less than five hours at 25°C., is quickly destroyed by ether, benzol and chloroform, and will not dialyse readily if at all.

In this ready destruction of photogenin by the fat solvent anaesthetics, the fire-fly resembles *Pholas dactylus* and differs markedly from *Cypridina*. One sample of *Cypridina* photogenin was preserved under chloroform for over 54 days and still gave light with photophelein.

Luciola photogenin is destroyed at about 42°, while the photophelein is still active after ten minutes boiling. A bright light is produced on mixing the two at 0°C.

Very weak concentrations of acids prevent the production of light of the fire-fly, less weak concentrations of alkalis are necessary and KCN does not affect light production in strong (m/100) concentrations.

The following table summarizes the characters of photogenin and photophelein (luciferase and luciferin) as found by Dubois for *Pholas* and by myself for *Cypridina* and the fire-fly. Where a statement runs through both photogenin and photophelein column it applies to a mixture of the two substances. A blank indicates that the experiment has not been tried.

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STUDIES ON BIOLUMINESCENCE

VI. LIGHT PRODUCTION BY A JAPANESE PENNATULID, CAVERNULARIA HABERI

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Contribution from the Physiological Laboratory, Princeton University, and the Department of Marine Biology, Carnegie Institution of Washington

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Panceri (1), working with *Pennatula phosphorea*, has given us the best account of phosphorescence among the pennatulids. He describes the light as coming from eight bands of cells on the outer wall of the stomach and continued into buccal papillae. They contain a luminous "fatty" matter which can easily be squeezed out as a sort of slime and which does not decompose readily. He also found that on stimulation a wave of light would pass over the colony in any direction at a rate of about 5 cm. per second. In a form like *Cavernularia* he describes also somewhat similar conditions (1, p. 40), but his descriptions are very meager. I have found the Japanese species of *Cavernularia* more favorable for light experimentation than *Pennatula* and the following pages contain confirmation of Panceri's experiments on the light waves passing over the colony and a general account of the chemistry of light production.

GENERAL CHARACTERS

Cavernularia haberi is especially abundant in the fjord of Aburatsubo, Japan, near Misaki, the Marine Station of the Imperial University of Tokio. The colony of animals lies hidden in the sand and contracted during the day, but at night takes up water and expands, large ones to the length of 2 feet. If stimulated by touching or electrically or by the addition of ammonia, a slime is formed similar to that produced by most of the *Cnidaria* upon irritation, but differing in that it is brightly luminous. The whole of the outer surface of the colony can form the luminous slime, but not the spongy inner material. The stalk, containing no polyps, is especially brilliant.

The slime may be dried over $CaCl_2$ and will give light when moistened with sea water or fresh water.

By squeezing *Cavernularia*, from which most of the sea water has been gently pressed, one can easily obtain a luminous juice which is still luminous when filtered through filter paper and retains its luminescence for several hours. Examination of the filtrate under the microscope in the dark shows that the light comes from minute points of light which makes the field of view look like the starry heavens. If water is added to this sea water juice, the light is greatly increased due to the appearance of numerous additional points of light. These points of light come from minute granules and globules easily visible in the filtered juice under the microscope. The addition of water to a dark *Cavernularia* juice which has stood for two days will cause the appearance of light due to the dissolving of the granules and globules. So sensitive are they to the addition of water that one drop of fresh water added to 5 cc. of juice will produce light, and so bright is the light that the addition of one drop of *Cavernularia* juice to 5 cc. of fresh water produces a light easily visible when the drop is mixed with the 5 cc.

By centrifuging the filtered juice, the granules may be partially thrown down and will give a brilliant light if fresh water is added while the liquid itself (turbid in appearance) gives a much fainter light. No light is produced upon the addition of water to a parchment paper sea water dialysate of the juice, so that light production is undoubtedly connected with the visible globules and granules of the juice. That it is connected with the solution of the granules is indicated by the fact that fresh water but not salt water or cane sugar is able to call forth the production of light. The process appears to be similar to the cytolysis of cells as can be observed by an inspection of table 2 which shows the effect of adding various substances to the *dark* juice of *Cavernularia*.

The light giving granules of *Cavernularia* will pass through an alundum filter crucible (R A 84) of the finest pores but not through a Pasteur-Chamberlain filter tube. The liquid passing through the latter is perfectly clear and non-luminous and gives no light when water is added.

There is no adsorption of the light producing substance by bone black or $Fe(OH)_3$.

ELECTRICAL STIMULATION

The juice of *Cavernularia* filtered through filter paper does not respond to the strongest interrupted induced shocks. The living colony, however, responds readily.

When a galvanic current is passed through one of the excised polyps mounted between non-polarizable electrodes, a flash of light occurs on the make and a series of flashes while the current is passing, which cease on the break. There is no flash of light on the break. A similar response can be observed with *Noctiluca* (2). It will be remembered that Romanes (3) observed a series of contractions in the bell of a medusa during the passage of a galvanic current, and the sartorius muscle of the frog often contracts on the make of a galvanic current, remains contracted during the passage of the current and relaxes on the break.

If the whole colony be stimulated by weak induced shocks, there is a local production of light. There is usually no response to a single shock, but a ready response to three or more sent in in rapid succession. With stronger stimuli, a wave of light, easily followed by the eye, passes over the colony in each direction from the point stimulated. With interrupted induced shocks, a series of waves of light follow one another in quick succession (not corresponding to the number of stimuli, however), reminding one of the series of electric shocks given out by the torpedo, only on a slower scale. The time interval between separate flashes no doubt corresponds to the refractory period of the cells concerned.

On pressing deeply into the tissue and stimulating strongly, a much brighter light response also results which very slowly moves away from the point of stimulation and usually does not extend more than 2 or 3 cm. At the same time the whole colony contracts, the polyps are drawn in and in this condition do not respond to electrical stimulation by light production.

The wave of light above mentioned will pass in any direction over the colony and across a cut around the middle of the colony, involving the whole of the external tissue. Some inner tissue must therefore be capable of conducting the stimulus.

LACK OF OXYGEN

That the production of light by *Cavernularia* is an oxidation can be very readily determined by passing a current of hydrogen through the juice, when the light disappears but promptly reappears upon admitting oxygen. A sample of phosphorescent juice kept twenty-four hours in an atmosphere of hydrogen gave light when air was admitted.

Considerable oxygen is necessary as may be seen by keeping the juice in a tall test tube, when it lights only at the surface in contact with air. The light appears throughout the tube, however, if mixed with air.

REDUCTASE, OXIDASE AND CATALASE

A tube of *Cavernularia* juice shut off from the air very quickly reduces (decolorizes) methylene blue. The blue color reappears when oxygen is admitted. This reaction has nothing to do with light production, however, as it is given also by a non-luminous sea anemone (Anthopleura xanthogrammica) and many other animal tissues which can not produce light.

Unboiled *Cavernularia* juice also gives oxidase reactions upon addition of H_2O_2 with guaiac, pyrogallol (slight), a-napthol, para-phenylen diamine, indophenol reagent, ortol and pyrocatechin. The boiled juice gave a slight positive test with guaiac, para-phenylen diamine and the indo-phenol reagent. Many other non-luminous tissues give these reactions, however, and there is abundant evidence to show that light production in animals is not connected with the ordinary oxidases (peroxidases) (4).

As in all other organic tissues, catalase is also present in the juice of *Cavernularia*.

TEMPERATURE

The juice of *Cavernularia* still gives a good light at 0°C., as do pieces of the colony if stimulated.

On raising the temperature, the light of *Cavernularia* juice disappears at 52° and does not reappear on cooling. Pieces of the colony slowly heated begin to light spontaneously at about 40°.

The light of *Noctiluca* (2) disappears at 48°, *Cypridina* (5) at about 54°, a hydroid, *Sertularia* sp. at 54°, the fire-fly, *Luciola* (6) at 42°, and luminous bacteria at 38° (8).

PHOTOGENIN AND PHOTOPHELEIN

Unlike the fire-fly, *Cypridina* and *Pholas dactylus*, it is impossible to separate the luminous juice of *Cavernularia* into two substances, photogenin and photophelein (luciferase and luciferin) (6), one destroyed by boiling, the other not, which will give light when mixed

(4, 5). We can not, for instance, cause light to appear in *Cavernularia* juice which has stood until the light has disappeared by adding fresh luminous *Cavernularia* juice heated to boiling and then cooled; neither can we obtain light by adding juice heated to temperatures below boiling (88°, 81°, 71°, 61° or 52°C.) and then cooled.

Another species of Pennatulid, *Pennatula* sp., as also *Noctiluca* and the squid, *Watasenia scintillans* behaves as does *Cavernularia*. None of these organisms gives the photophelein-photogenin (luciferin-luciferase) reaction for reasons at which we can only guess. The photogenin or photophelein may be, either of them, very unstabile or there may be sufficient photophelein to use up all the photogenin. The evidence in this case seems to indicate that the photophelein is unstabile as we can obtain a faint light with *Cypridina* photophelein or fire-fly photophelein (both prepared with sea water) and the non-luminous *Cavernularia* juice (photogenin). *Pennatula* and *Noctiluca* photophelein gave negative results with *Cavernularia* juice and vice versa.

The question may be asked—what substances are able to cause light to appear in the juice of *Cavernularia* which has stood until completely dark? Usually four to six hours are sufficient time for the juice to lose its luminescence. It is still capable of giving out a bright light if we add fresh water (but not sea water) to it, and it retains this potentiality for over two days at 20°C. (see table 1) and a shorter time at higher temperatures. As only fresh water and not sea water will call forth the light, and as we know that light production is connected with the granules of the juice, the process appears similar to the cytolysis of cells—i.e., to the swelling and solution of the granules which the cells contain, in fresh water.

By this means something is liberated from the granules of *Cavernularia* juice which oxidizes with light production. Table 2 gives the results of adding various other substances, pure or dissolved in sea water, to the dark *Cavernularia* juice. Note from the table that

TABLE 1

Effect of standing on light producing power of Cavernularia juice

Juice + water	Bright light
Juice 12 hours old at 20°C. + water	Bright light
Juice 24 hours old at 20°C. + water	Fair light
Juice 48 hours old at 20°C. + water	Faint light
Juice 72 hours old at 20°C. + water	None

TABLE 2

Effect of substances in calling forth light from non-luminous cavernularia juice

SOLUTION OR SUBSTANCE ADDED	CHARACTER OF LIGHT
50 per cent sea water	Bright light
66 per cent sea water	Fair light
75 per cent sea water	Fainter light
80 per cent sea water	Very faint light
90 per cent sea water	None
Water	Bright light
Sea water	None
Sea water evaporated to one-half volume	None
m cane sugar	None
m/2 cane sugar	Faint light
m/4 cane sugar	Fair light
m/8 cane sugar	Good light
Chloroform saturated sea water	Faint light
One-half Chloroform saturated sea water	Very faint light
One-quarter Chloroform saturated sea water	None
Chloretone saturated sea water	None
One-half Chloretone saturated sea water	None
One-quarter Chloretone saturated sea water	None
Thymol saturated sea water	Fair light
One-half Thymol saturated sea water	Faint light
One-quarter Thymol saturated sea water	Very faint light
Benzol	Faint light
Ether	None
Chloroform	None
Chloretone crystals	Very faint light
Thymol crystals	Faint light
Chloral hydrate crystals	Faint light
Saponin powder	Fair light
Oleic acid	Faint light
Ortol crystals	Faint light
Hydrochinon crystals	None
Resorcin crystals	None
Pyrocatechin crystals	None
Pyrogallol crystals	None
Ba_2O_2 powder	None
Na_2O_2 powder	None
$KMnO_4$ crystals	None
$K_2Cr_2O_7$ crystals	None
$FeCl_3$	None
$FeSO_4$ crystals	None
$K_4Fe(CN)_6$	Faint
Laonome japonica (an annelid) blood	None
Dried ox blood extract in sea water	None
Lepas anatifera extract	None

TABLE 2—Continued.

SOLUTION OR SUBSTANCE ADDED	CHARACTER OF LIGHT
Lepas anatifera extract boiled	None
Chiton sp: extract	None
Chiton sp: extract boiled	None
Onchidium sp. extract (a pulmonate mollusc)	None
Onchidium sp. extract boiled	None
Dolabella sp. blood (a nudibranch)	Faint
Dolabella sp. blood boiled	None
Sepia esculenta blood	None
Sepia esculenta blood boiled	None
Panulirus japonica blood	Very faint light
Panulirus japonica blood boiled	None
Cypridina hilgendorfii photophelein in sea water	Fair light
Luciola vitticollis photophelein in sea water	Fair light

light production is not due to dilution of the salts of sea water by adding fresh water, since m cane sugar does not call forth the light. Note also that many cytolytic substances (chloroform, benzol, thymol, etc.) give light, but not the oxidizing agents—Na_2O, $KMnO_4$, etc. The blood of certain invertebrates also causes very faint light production, but we can not be sure that this is not due to the fact that the blood is somewhat less concentrated than sea water, although the determinations of other closely allied forms show the salt content to be the same as the sea water in which they live.

CHEMICAL REACTIONS

The luminous juice of *Cavernularia* contains the luminous substance but mixed with many other substances so that mere chemical tests on the juice are of no value in determining the chemical nature of the luminous substance. The luminous material is salted out along with the other proteins but does not retain its power to phosphoresce long enough to be manipulated by the ordinary chemical methods. The following paragraphs give the results of precipitation by $(NH_4)_2SO_4$, picric acid, alcohol, etc.

If to the fresh filtered luminous juice of *Cavernularia* we add sugar, NaCl, $MgSO_4$, $(NH_4)_2SO_4$ to saturation, or 5 volumes of glycerine, the light disappears. A heavy precipitate forms in $(NH_4)_2SO_4$), a small precipitate in $MgSO_4$ and practically none in NaCl. The precipitates are soluble in sea water. No precipitate is formed in sugar or glycerine. If poured into fresh water or sea water immediately after the light has disappeared, light is produced by all solutions. If the tubes are allowed to stand for a day, no light is produced by any tube on pouring into fresh water or sea water.

If alcohol or acetone (3 vols.) is added to the juice, a precipitate forms and the light disappears. The precipitate is insoluble and gives no light in sea water or fresh water.

The alkaloidal reagents, picric acid, phosphotungstic acid and tannic acid immediately cause precipitation with disappearance of light. The precipitates are insoluble in sea water or fresh water and produce no light.

Normal acetic acid or n NH_4OH, added drop by drop produce no precipitate although the light disappears in weak concentrations.

ANESTHESIA

Ether, chloroform and benzol cause a fairly rapid disappearance of the light in the order named, the ether most rapid. In turpentine, chloral hydrate, thymol and chloretone, the light stays for a long time and then disappears. There is no reappearance of light from any of the tubes if poured into fresh water or sea water unless we do so immediately the light has disappeared, and then the light is very faint and momentary.

In other words, the juice can not be anesthetized. Even in concentrations of alcohol, ether, chloroform or butyl alcohol which cause a slow disappearance of light, there is no recovery or only a momentary faint glow upon dilution of the solutions with sea water. The alcohols give similar results. About 16 per cent ethyl alcohol will cause the light to disappear in one minute, but there is no recovery or only a very faint momentary glow on diluting with sea water. Saturation with butyl alcohol gives a similar result.

Since reversibility is the *sine qua non* of anesthesia, we must conclude that this juice can not be anesthetized, in this respect differing markedly from the granule free *Cypridina* luminous secretion which can be anesthetized by the alcohols. Also many luminous organisms, for instance luminous bacteria (9), can be truly anesthetized.

POTASSIUM CYANIDE

Potassium cyanide has no inhibiting effect upon the light production of *Cavernularia* juice. Even in m/40 concentration, the light is still bright after ninety minutes, a result agreeing with all my other experiments on luminous organisms (bacteria, fire-fly, *Cypridina*, *Noctiluca*).

SUMMARY

1. The light of *Cavernularia* comes from a luminous slime, a secretion of gland cells over the outer surface of the colony.

2. The secretion contains small granules which can be seen to emit the light. On standing the light from a sea water extract of *Cavernularia* slowly disappears and will not return on shaking, but reappears if fresh water is added to the juice.

3. The light substance (i.e., the granules) will pass an alundum filter (R A 84) but not a Chamberlain filter. It is not adsorbed by bone black or $Fe(OH)_3$ and will not dialyse.

4. The light producing granules do not respond to electrical stimulation but the colony gives light with galvanic or induced currents.

5. With galvanic currents a flash appears on the make and a series of flashes while the current is passing which cease on the break.

6. Upon stronger stimulation (with induced currents) a wave of light passes over the colony in all directions from the point stimulated. This wave will pass through the deeper tissues when all the ectodermal tissues are cut.

7. A considerable amount of oxygen is used up in light production and no light appears in its absence.

8. *Cavernularia* juice will decolorize (reduce) methylene blue in absence of oxygen and it also contains peroxidases and catalase.

9. The light is still bright at 0°C. Fragments of *Cavernularia* give off light spontaneously at 40° which disappears at 52°C. and does not return on cooling.

10. The photogenin-photophelein reaction is not given by *Cavernularia*, but a faint light can be obtained with a non-luminous *Cavernularia* juice (photogenin) and *Cypridina* or fire-fly photophelein.

11. The production of light by the granules appears similar to the cytolysis of cells as it occurs with water (but not isotonic cane sugar) and certain cytolytic substances (saponin, chloroform, benzol, oleic acid).

12. The light producing substances are salted out along with the proteins but are not stabile enough for chemical manipulation.

13. The light producing granules can not be anesthetized.

14. Potassium cyanide has no effect on light production.

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RESEARCHES ON THE EXCHANGE OF ENERGY IN LIVE ANIMAL TISSUES

II. Study on the Liver

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I. INTRODUCTION

The technical difficulties surrounding the obtaining of exact and direct measurements of small amounts of heat developed in large periods of time, have prevented the conclusion of the chapter of physiology on animal heat (which, as regards the animal itself, whole and intact, has progressed considerably and is at present in a high stage of perfection), as such a conclusion depends on the analysis of each organ, and each separate tissue, of the system to which they belong, in order to determine, once for all, the rôle of each organ or tissue in the total production of heat. These difficulties, unsurmountable until quite recently, on the one hand, and the importance of the problem on the other, induced physiologists to attempt measuring the heat produced by each organ, and, in many instances, they were content with just an idea, more or less approximate, of this production, obtained by indirect and extremely defective methods. They had recourse, in some cases, to mere thermometrical readings; and, in others, to the valuation of the amount of heat generated, using as a basis the amount of O_2 absorbed or of CO_2 exhaled.

The first method may lead us to admit, at the most, that an organ which becomes much heated (as a muscle in operation), or the temperature of which is maintained above that of its environment (as happens with the liver), also generates much heat; such a method however, does not make it possible to determine absolutely the amount of heat generated. The second method would appear, on a cursory analysis, to be satisfactory. It comprises a double operation: (1) the determination of the amount of respiratory exchanges; and (2) the indication of the thermical coefficients of CO_2 and O_2, by which should be multiplied the figures obtained by the first operation, in order to ascertain the heat generated.

The respiratory exchanges of an organ left "in situ" and with all its relations (nervous, vascular and so forth) intact, could be determined by measuring the total amount of gases in the blood before and after passing through such organ; in view, however, of the uncertainty of blood irrigation, which constantly varies, and the equal oscillation of the intensity of respiration itself, this operation would require continuous, or almost continuous, sampling of arterial and venous blood and, furthermore, the exact knowledge, during the experiment, of the total quantity of blood which passed through the organ. The mere statement of these conditions shows us the difficulty—one may almost say the impossibility—of obtaining precise results. But, even admitting that it were possible to obtain the exact amount of O_2 and CO_2 absorbed by the organ under experiment, the fact that all its organic (nervous, vascular, etc.) connections were intact, might occasion a mistake in the valuation of the heat generated, as the total quantity of chemical energy released by the absorption of O_2 may not be transformed, directly and immediately, into heat but into another form of intermediary energy which, passing on to another organ, would then be turned into heat. Let us suppose, for instance, that a part of the chemical energy supplied to the cerebrum by the combustion of food, is released by it for the purpose of generating impulses which are passed on to the muscles; the valuations of heat, of the cerebrum or of the muscles, taking as a basis the respiratory exchanges, would bring us to figures which would be excessive for the cerebrum and too small altogether for the muscles. These hypotheses may not become true, but the mere possibility of their occurring is sufficient to cause mistrust and doubt of the value of respiratory calorimetry under such conditions, and makes it imperative for us, in order to simplify the study of the matter, to work with isolated organs, separated from all and any organic connection.

Once the value of respiratory exchanges is known, that is to say, once the quantity of O_2 consumed, and of CO_2 generated, have been ascertained, it is necessary to multiply one of these numbers (let us say O_2, for instance) by its thermical coefficient.[1] But, even then, in order that the amount of heat calculated may be as near the real figure as possible, it is advisable to determine the nature of the alimentary substances burned; this problem is solved by respiratory calorimetry applied to an animal, taking into account, during the experiment, its condition of nourishment or fasting, the nature of the food absorbed (protein, fats or carbohydrates), the respiratory quotient of Pflüger. When, however, one is dealing with isolated tissues, these indications of the nature of the food potentials burned lose their significance and all that remains is the respiratory quotient, which, by itself, does not enable one to determine the nature of the food, because its present value may be the result of the simultaneous combustion of various substances. Then, again, one knows that the animal receives food always in the same initial condition (protein, fats, and carbohydrates) and abandons them in the same condition—CO_2, H_2O and urea—or bodies very much the same from a thermo-chemical point of view; the amount of heat generated by the transformation of one molecule of each of these substances depends, therefore, on its initial as well as on its final condition, and the respective thermical coefficients of O_2 are also an exclusive function of the said initial and final conditions besides depending, of course, on the very nature of the substance transformed. Now, between the initial condition and the final condition of one of these foods, many intermediary phases occur, the majority of which are, as yet, unknown. If, therefore, these phases occur in different organs, it follows that the thermical coefficients will also vary from organ to organ, and thus the respiratory calorimetry applied to isolated parts of the organism would induce one into ever increasing error. For instance: If the total transformation of fat into CO_2 and H_2O by combustion, takes place, as is most likely, in two phases: the first of the fat into glycose in the liver and the second, from glycose into CO_2 and H_2O in other tissues, as for instance, in the muscles, the amounts of heat developed by each of these phases will be different from each other, and the thermical coefficient to be used in each case will be that of fat for the entire organism, that of glycose for the muscles, and that of the transformation of fat into glycose for the liver, on the value of which there are still some doubts.

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[1] The thermical coefficients of O_2 instead of those of CO_2 are recommended because the thermical coefficients of O_2 have the great advantage of being much less variable according to the nature of the food burned.

In the above example, however, we have taken it for granted that the initial and the final conditions of the transformation in each organ are already known, and, of course, if they are not known, the uncertainty will be but increased. In short, one may say that just because respiratory calorimetry may render it possible to calculate the amount of heat generated by an animal, it does not follow that it is also possible to calculate thereby the amount of heat generated by each organ or each tissue of the said animal; the problem, in the latter case, is indefinite, and the probabilities of error in these indirect respiratory valuations are so many that more direct measures are indispensable.

In this paper we purpose: (1) Measuring the amount of heat developed by the liver; (2) measuring its respiratory exchanges; (3) establishing the value of the thermical coefficients of O_2 and CO_2 for the liver.

STUDIES ON THE LIVER

We have made use of the technique studied in our preceding paper (1). As we then saw, the tissues are reduced to small fragments, so as to considerably increase their surface of respiratory exchange with their environment. This is the application of the principle used by Battelli and Stern in their experiments on the respiration of tissues. These authors (2) demonstrated that, even after total destruction of the cellular structure, the fragments of cells and the liquids from same were still able for some time to absorb O_2 and eliminate CO_2. It is easy to understand, however, under conditions so entirely abnormal, the results obtained could hardly be applied to the case of normal organs; it was, therefore, necessary for us to make sure that our grinding apparatus would respect the integrity of the great majority of the cells, acting as a mere mechanical separator of the organ; to make sure of this, which otherwise might eventually prove an objection to the technique adopted, we asked Prof. Raul Leitão de Cunha, teacher of anatomy and pathological physiology of the Rio de Janeiro Academy of Medicine, to make a microscopic examination of the liver emulsions used by us.

The following is the report of the Professor, to whom we are exceedingly grateful:

> The histological analysis of the matter submitted resulted in the identification or tracing of the following elements:
>
> (1) A large number of deformed blood corpuscles (hematias) which, in view of their number, constituted the predominant element.

(2) Hepatic cells distributed according to the following types:
  (a) Detached and well shaped, as regards nucleus and protoplasm.
  (b) Together in pairs, and in the same morphic conditions as above.
  (c) In single file, well shaped, with up to six cells per string.
  (d) Grouped in cords of two or three strings (as described in letter c) joined together.
  (e) Forming large spots or plates, but one layer deep thus allowing the verification of their anatomic integrity.
  (f) In more or less voluminous masses, in which their connections with each other are maintained intact.
  (g) Detached or agglomerated, but broken up into fragments more or less difficult to identify.
(3) Groupings of leucocytes, mainly polymorphous-nucleated.
(4) Remainders of the Glisson capsule, joined in masses of various sizes.
(5) Vascular débris and others, forming amorphous agglomerates, difficult to penetrate with the light of the microscope.

It is evident from the above that the liver emulsion used in the experiments which we shall proceed to describe contains, as a rule, cells either completely detached or in groups small enough to prevent asphyxiation and to allow their easy respiration.

The following table condenses the result of the experiments made on the liver in pursuance of the technique mentioned before.

Table showing the result of experiments made on liver emulsion

ORDER NO.	CO_2	O_2	$\frac{CO_2}{O_2}$	HEAT GENERATED (Q)	THERMICAL COEFFICIENT OF O_2—$\frac{Q}{O_2}$	THERMICAL COEFFICIENT OF CO_2—$\frac{Q}{CO_2}$
Experiment I......	347.4	330.0	1.05	2639.2	7.99	7.59
Experiment II......	468.2	418.0	1.12	2633.2	6.29	5.62
Experiment III......	529.6	436.0	1.21	3370.6	7.72	6.36
Experiment IV......	353.6	303.4	1.16	2306.0	7.60	6.52
Experiment V......	506.1	390.0	1.29	2253.0	5.77	4.45
Experiment VI......	355.8	324.9	1.09	2099.0	6.46	5.89
Average............	426.7	367.0	1.15	2550.1	6.97	6.07

NOTE. CO_2 and O_2 are reduced when dry and at a pressure of 760 and 0°; the figures shown in the above table represent cubic centimeters and refer to the respiratory exchanges of 1 kilo of liver during one hour.

The heat is represented in gram-calories per kilo-hour.

The results obtained are quite different from what might be expected. The thermical coefficients of O_2 and CO_2 can not be connected with any of the coefficients relative to the common alimentary substances—

protein, fats or carbohydrates, and even less to the thermical coefficients of transformation of the nutritive reserves, which develop a lesser amount of heat. Furthermore, if the average of the coefficients of O_2 (6.97) is greater than the thermical coefficient of all the transformations known to take place in the organism, it is also too great for any of the intermediary phases. We are, therefore, led to admit either that the amount of heat determined by us is excessive and wrong, or else that the measurement of the gases resulted in figures altogether too small for O_2. We are positive that the measurements of heat are rigidly conducted and that the results really represent the actual facts, as may be verified by our first paper, and by a thorough discussion of the method employed. As to the measurement of the gases, however, we must confess to great doubt as to their accuracy. Compared with the figures of Battelli and Stern (3), the absolute values of our readings are lower, but this is probably due to the fact that, in their experiments, these authors submitted the tissues to a much more energetic agitation than we did, and also to the fact that the tissues breathe in an atmosphere of pure oxygen, and not in the common atmospheric air. We have, indeed, remarked that, towards the end of each experiment, the emulsion of tissues in defibrinated blood presents a more or less dark or venous color, although there are still notable quantities of O_2 in the atmosphere of the bottle, which would indicate slowness in the diffusion of O_2 and of its absorption by the tissues. On the other hand, however, the value of the respiratory quotient $\frac{CO_2}{O_2}$ is similar to that obtained by Battelli and Stern, for the liver.

In our experiments, as in the majority of the experiments of these authors, the relation $\frac{CO_2}{O_2}$ has very high values, exceeding unity. Now, were this true it would mean, most assuredly, a transformation of carbohydrates into fats, in the liver, detached or isolated, an extremely interesting thing, but one which is neither plausible nor true, as we shall later demonstrate. Comparing this with the exclusive value of the thermical coefficient of O_2, we are led to admit that our measurements of the gases, taken as they were, notwithstanding, with considerably more care and rigidness than by the gross and inexact methods of Battelli and Stern, yet contain causes of error which it would be advisable to obviate. With this in view, we have originated the following technique for the measuring of gases.

PROCEDURE FOR MEASURING RESPIRATORY EXCHANGE OF TISSUES

Up to now, in endeavoring to determine the proportion of the respiratory exchanges of tissues, we used the following procedure: A sample was taken of the air in the calorimeter and submitted to eudiometric analysis, the carbonic gas then found, or the deficit of O_2 registered, being considered by us as representing faithfully the respiratory exchanges of the tissues, just as if they had breathed directly in the air. This is the method followed by Battelli and Stern in many of their experiments; in others, however, where the tissues breathed in an atmosphere of O_2, a simple manometric measurement showed the decrease in the volume of O_2 in the breathing chamber and, consequently, the amount of O_2 absorbed.

We shall now consider the tissue breathing in defibrinated blood, or even in the suspension of red globules in Locke's fluid, which we have designated as "artificial blood." The oxygen consumed by the tissues is absorbed from the O_2 contained in the artificial blood, in dissolution or combined with the hemoglobin of the red globules; as the O_2 in the artificial blood diminishes in consequence of this operation, a new quantity of O_2, drawn from the gaseous atmosphere of the calorimeter, is then absorbed. There is, however, as is well known, no simple relation of equality between the amount of O_2 consumed by the tissues and the amount of O_2 drawn from the atmosphere of the calorimeter, no matter how intensely the tissues are agitated. On the other hand, the CO_2 in the air of the breathing chamber represents the addition of two parts: one produced by the tissues during the experiment, and the other (which is most variable in volume) already existing in the tissues and in the defibrinated blood when placed in the calorimeter. One may, therefore, state that the variation in the composition of the atmosphere of the calorimeter does not determine the amount of respiratory exchanges during the experiments.

To attain this end, it is necessary to proceed, in each case, to two measurements of the gases, one at the beginning, and one at the end of the experiment, so as to obtain, by difference, the real value of the respiratory exchanges; furthermore, each operation should be so made as to determine simultaneously the amount of the gases in the atmosphere of the calorimeter and that of the gases combined or dissolved in the emulsion of tissues. The problem is, therefore, rather a complex one and, to simplify it, we release the gases of the emulsion, add them to the gases which form the atmosphere of the calorimeter, and proceed with the operation for determining the totality of gases of this mixture, which represents the entire quantity in experiment. To obtain the gases in the blood and in the tissues, the emulsion is submitted to treatment by a 20 per cent solution of phosphoric acid, to free the CO_2 and by a solution of ferri-cyanid of potassium, less than two months old, to free the O_2 by transformation of hemoglobin into methemoglobin. In other words, we use Haldane's method of chemical analysis of gases of the blood (4). Our experiment made it clear that these two solutions could be mixed and used simultaneously; the transformation of hemoglobin into methemoglobin by the ferri-cyanid solution takes place even more rapidly with the addition of phosphoric acid, than without it, and, on the other hand, this chemical substance absolutely does not prevent the release of the CO_2 in the blood by the phosphoric acid. This above method does not give absolute value of the quantities of gas; but, in a comparative analysis, the results obtained by difference are extremely precise.

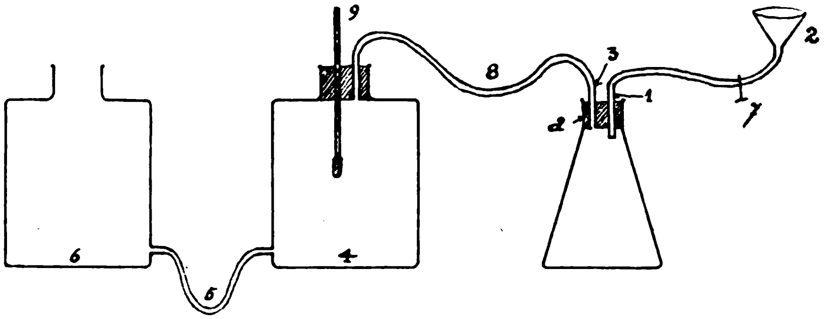

FIG. 1

To apply to our case the general principles of chemical analysis of the blood gases, we originated the technique which we shall proceed to describe. The emulsion of tissues is prepared in double the quantity required for the calorimetric experiment; it is then divided into two equal parts, brought to the same temperature (see memo.) and, at the right moment, a part is poured into the calorimeter for the experiment, and the other is placed in an Erlenmeyer bottle, of the same capacity as that of the calorimetric receptacle. This bottle has a stopper, (*a*) through which pass tube (*1*) connected with funnel, (*2*), and tube (*3*), connected by a rubber tube, (*8*), to receptacle, (*4*), which, in turn, is connected by a rubber tube, (*5*), to receptacle (*6*). Receptacles (*4*) and (*6*) form, therefore, a system of intercommunicating vessels, in which is placed mineral oil (which will not absorb the gases) in such quantity that, when receptacle (*6*) is raised conveniently, the oil will completely fill receptacle (*4*), this being their initial position, and so that, when both receptacles are placed on the same horizontal plane (in such a way that the level of the liquid in each bottle will be equal) the volume of the gases in receptacle (*4*) will be known and constant, equal, for instance, to 1000 cc.[2] The emulsion of tissues having been placed in the Erlenmeyer bottle, this is closed with stopper (*a*), the pressure cock (*7*) is opened and the mixture of ferri-cyanid and phosphoric acid contained in funnel (*2*) is allowed to flow down. During all this time, the bottle should be constantly agitated. These liquids, besides releasing the gases of the emulsion, will immediately cause its respiration to cease, not only on account of the powerful acidity they will originate, but also in view of the toxic action of the ferri-cyanid of potassium.

The excess of gases will then begin to pass into receptacle (*4*), reducing the level of the oil contained therein. When all the gases of the emulsion are released (say in ten minutes),[3] the remaining gases, contained in the Erlenmeyer bottle, are forced out by merely filling the bottle completely with mineral oil through funnel (*2*). Then, with receptacles (*4*) and (*6*) on the same horizontal plane, rubber tube (*8*) is withdrawn from glass tube (*3*), and the latter is allowed to communicate with the atmosphere.[4] When this is done, the oil in receptacle (*4*) will fall until it is on the same level with the liquid contained in receptacle (*6*), whilst, at the same time, it will draw in a certain amount of air from outside.

We shall then have in receptacle (*4*) a known volume of air, equal, for instance, to 1000 cc. at atmospheric pressure and at the temperature registered by the thermometer (*9*). This volume of air will contain all the gases of the emulsion of tissues, plus an unknown quantity of atmospheric air, (part, air already contained in the Erlenmeyer bottle, and, part, air drawn in from the atmosphere). When the calorimetric experiment is finished, the same operation is repeated for the suspension of tissues, substituting the Erlenmeyer bottle by the calorimetric receptacle, the stopper of which, as we have already seen, is also crossed by two glass tubes. Thus we have a second gaseous mass, modified in its composition by the respiration of the tissues, during the calorimetric experiment. We then make an eudiometric analysis of these two gaseous volumes, which we may call:

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[2] This volume should considerably exceed that of the calorimetric receptacle, or that of the Erlenmeyer bottle.

[3] We always allow the same length of time for the release of the gases from the emulsion—ten minutes, during which time the bottle is strongly agitated.

[4] As tube (*8*) is a long thin tube, and during this phase of the experiment the pressure in receptacle (*4*) is less than the atmospheric pressure, there is no fear of losing the gases contained in receptacle (*4*).

(A) The first, or the one indicating the amount of gases in the tissues, when they were placed in the calorimeter; and

(B) The second, showing those found at the conclusion of the calorimetric experiment.

The results, when dry, are reduced, at a pressure of 0°, 760 mm. and with these data we are able to determine the quantities of O_2 and CO_2 breathed during the calorimetric experiment, as we shall proceed to demonstrate.

By the first analysis (A), we obtain the total quantity (Q) of O_2 existing in the said volume; (Q) being equal to the sum of two items, namely, the first (Y_1) representing the volume of O_2 originally existing in the Erlenmeyer bottle, and in the air drawn in from the atmosphere by receptacle (*4*), to complete the final volume of (A), as stated above; the second (Y_2), representing the volume of O_2 existing in the emulsion of tissues, either in dissolution, or combined with the hemoglobin of the blood.

We have, therefore:

$$Q = Y_1 + Y_2 \qquad \text{(A)}$$

As (Q) is known, from the eudiometric analysis, if we also knew (Y_1) we would establish, by difference, the quantity which we are endeavoring to ascertain (Y_2).

By the second analysis (B), we find that the quantity (Q′) of O_2 measured, is equal to the quantity (X_1) of atmospheric O_2 which existed in the calorimeter at the beginning of the experiment, or which was drawn in by receptacle (*4*), plus the quantity originally existing in the emulsion of tissues, deducting from the total of this addition the amount of O_2 breathed by the tissues (X_2) during the experiment. We have, therefore:

$$Q' = X_1 + Y_2 - X_2 \qquad \text{(B)}$$

By this system of equations, (Q) and (Q′) are obtained by the experiment, and (Y_1) and (X_1) may be calculated if we take as a basis the following observations:

In the first place: Y_1 and X_1 (the O_2 being drawn in from the surrounding atmosphere, or having entered, from the atmosphere, into the calorimeter or into the Erlenmeyer bottle) are accompanied by such a quantity of nitrogen that the relation $\frac{O_2}{N}$ is constant; thus, in the open air, this relation is equal to 0.264 and holds true with remarkable stability even in the most variable climates; in our laboratory, where the windows are always open into a garden, this relation is constant; however, if the operations are conducted in a confined atmosphere, there is no reason why the necessary steps should not be taken to determine the value of $\frac{O_2}{N}$ in the atmosphere where the experiment is made.

In the second place: nitrogen is chemically indifferent to reactions which take place in the tissues, and its quantity remains unchanged. Now, analyses (A) and (B) indicate the quantities (N) and (N′) of nitrogen contained therein, and the quantities (Y_1) and (X_1) of O_2 are, therefore, given by the following expressions:

$$Y_1 = 0.264N$$
$$X_1 = 0.264N'$$

Substituting these values in the equations (A) and (B), we obtain the following system of two equations with two incognitas:

$$Q = 0.264N + Y_2 \qquad (A)$$
$$Q' = 0.264N' + Y_2 - X_2 \qquad (B)$$

taking the value of Y from (A) and substituting in (B), we have:

$$Q' = 0.264N' + Q - 0.264N - X_2$$

therefore: $$X_2 = 0.264(N' - N) + (Q - Q')$$

N and N′ are the volumes of nitrogen, and Q and Q′ are the volumes of O_2 determined in (A) and (B).

Therefore, the quantity of O_2 breathed by the emulsion of tissues is equal to the product of the relation between the O_2 and the nitrogen in the air of the laboratory, (as a rule 0.264) by the difference between the volumes of N determined in (B) and (A), plus the difference between the volume of O_2 determined in (A) and (B).

The nitrogen originally existing in the emulsion of tissues does not affect the results obtained, because its quantity, which depends exclusively on the temperature of the emulsion and on the pressure of the nitrogen in the outside air, varies but little, and is constant in the experiments (A) and (B); therefore, these quantities are added to both terms of the difference (N′ − N) without altering their values. The volume of CO_2, ascertained during the calorimetric test, is obtained by the difference between the quantity of CO_2 in (B) and the quantity of CO_2 determined in (A).

This is the technique which we are adopting in our experiments and, hereafter, unless advised to the contrary, such results as we may submit will be obtained by same.

As will be seen in this paper, the problem solved consisted in originating a technique for determining the amount of gases of the blood, and with this we obtained the results shown in the following table.

Table showing the result of experiments made on liver emulsion by following the new technique

ORDER NO.	CO_2	O_2	$\frac{CO_2}{O_2}$	HEAT GENERATED (Q)	THERMICAL COEFFICIENT OF O_2—$\frac{Q}{O_2}$	THERMICAL COEFFICIENT OF CO_2—$\frac{Q}{CO_2}$
Experiment I......	382.5	862.5	0.44	2473.0	2.86	6.46
Experiment II......	408.0	899.6	0.45	3520.0	3.91	8.62
Experiment III......	380.7	688.5	0.55	2686.7	3.90	7.05
Experiment IV......	318.0	756.2	0.42	2534.7	3.35	7.97
Average............	372.3	801.7	0.46	2803.6	3.50	7.52

Comparing this table with the previous one, it will be observed that the amounts of CO_2 obtained by the method derived from that of Batelli and Stern[5] are somewhat higher than they should really be. Where, however, a truly great difference may be observed—so great, indeed, that, in our opinion, it annuls the value of those experiments—is in the amounts of O_2 shown. As a consequence of this, the value of the respiratory quotient, which is very low and constant in signification in our last table, is, on the contrary, very high and of entirely different (and, one may say, opposite) signification in the preceding table.

These differences in the value of the gases measured naturally cause considerable variation in the thermical coefficients of CO_2 and O_2. It should be noted, however, that the calorimetric measurements, in any of this series of experiments, are valid and independent of the amounts of respiratory exchanges; if, in the first table, they are somewhat smaller this should be attributed exclusively to the influence of the environment in which the tissues breathe (namely, the suspension of red globules in Locke's fluid), as we have also remarked that the exchanges of gas are more active in defibrinated blood than in that environment. If we examine only the last table, the figures whereof are reliable, a few interesting facts will be observed: In the first place, the value of the thermical coefficients of O_2 or of CO_2, or the average of the values thereof, differ considerably from those that are used in the respiratory calorimetry; therefore, had we calculated the heat produced by the liver, multiplying by such values the O_2 consumed, the result obtained would have been widely different from the real figures. Then, the thermical coefficients obtained for O_2 are always very much lower, whether we consider each by itself, or take the average of the thermical coefficients of the alimentary substances (protein, fat or glycose). The thermical coefficients of CO_2 are, on the contrary, excessively high for these same alimentary substances. This coincides with (and is, in part, explained by) the extremely low value of the respiratory quotients, the average of which is but 0.46.

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[5] Only in regard to the manner of treating the tissues, as we measured the gases by means of an eudiometric glass, and not, as did those authors, by processes which are not reliable.

Comparing these different values of the respiratory quotients and those of the thermical coefficients of O_2 and of CO_2, we may, by a perfectly legitimate induction, infer, to a certain extent, the nature of given chemical reactions which are taking place in the liver. Thus we can state that in all our experiments, as shown in the aforesaid table, a pronounced transformation of fat into glycose or glycogen occurred in the liver. It is this transformation which explains, to a certain extent, the figures obtained. The liver works "in vitro," separated from the organism, and, therefore, from the pancreas, like a diabetic liver. The determination of the heat by the different organs "in vitro," and the measurement of the amounts of respiratory exchanges, by the aforesaid process, can therefore serve (and should serve) as a good method for the study of the nature of the metabolism of each organ, in its intensity and quality, and, consequently, should also serve for the study of the reciprocal action of the organs on each other.

In our next paper we shall go into the conditions which are necessary, in order to determine the nature of the combustions which occur in an organ, taking as a starting point the heat and the CO_2 and O_2 respectively produced and absorbed by it. At the same time we shall apply these principles to the study of the action of the pancreas on the liver.

CONCLUSION

1. The method employed by Battelli and Stern for determining the amount of respiratory exchanges of the emulsion of tissues, is fallacious, as the results obtained are erroneous.

2. A new technique is originated for the rigid measurement of respiratory exchanges of tissues suspended in defibrinated blood, which procedure is also applicable to the more simple process of determining the amount of gases in the blood.

3. We have determined the amount of heat, and the value of the respiratory exchanges of the liver separated from the organism.

4. We have demonstrated experimentally that with the data at present accepted in physiology, it is not possible to determine the amount of heat produced by the liver, by means of respiratory calorimetry.

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COMPARISON OF THE AMOUNT OF CATALASE IN THE MUSCLES OF LARGE AND OF SMALL ANIMALS

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The surfaces of two spherical bodies are to each other as the two-thirds power of their masses, hence the amount of surface per unit mass of the small body is greater than that of the large one. On account of this greater exposed surface the small body loses heat more rapidly than the large one. Similarly a small animal loses heat more rapidly than a large one, this greater loss of heat being made good by a greater production. Bergmann (1) was among the first to show that small animals require relatively more food and produce relatively more heat than large animals of the same species, that is the oxidative processes in small animals are more intense than in large animals. These facts have been expressed by Rubner (2) in the so-called Surface Area Law, according to which metabolism is proportional to the surface area or for the same amount of surface area there will be the same production of heat. Burge (3) showed that when oxidation is increased in an animal by increasing the amount of exercise, or decreased by decreasing the amount of exercise, the catalase content of the muscles of the animal is correspondingly increased or decreased. Hence, the conclusion was drawn that there is a close relationship between the amount of catalase in a muscle and the intensity of the intra-muscular oxidative processes. If such a relationship exists there should be more catalase in the muscles of small animals than in the corresponding muscles of large animals. The following experiments were carried out to determine if there is more catalase in the heart muscle of small animals than in the heart muscle of large animals. The heart was chosen because it was thought that the catalase content of this muscle would be less affected by the mode of life of the animal than any of the other muscles. The animals used in one series were the goose, pigeon and English sparrow; those in another, the dog, rabbit and rat. The geese were raised by a local farmer, the pigeons and English sparrows were birds accustomed to the range of the town, the dogs were tramp dogs, the rabbits were raised in captivity, and the rats were wild field rats. The catalase content of the hearts of these animals was determined according to the following method. The hearts were removed from the etherized animals and a cannula tied into the aorta with its point toward the heart. The cannula was then attached to a pressure bottle containing 0.9 per cent sodium chloride at 38°C. Thus the liquid was passed through the coronary vessels while the heart was still beating until the heart was washed free of blood. Each bloodless heart was ground up separately in a hashing machine and 1 gram of the material was introduced into a bottle containing 45 cc. of hydrogen peroxide and as the oxygen gas was liberated it was conducted to a burette previously filled with water. A full description of the method is given in a previous publication (3). After the oxygen gas, collected as described, had been reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in one gram of the hashed heart. The results of the determinations are given in tables 1 and 2.

TABLE 1

After heart, under goose, pigeon and English sparrow, are given the number of cubic centimeters of oxygen liberated in ten minutes from 45 cc. of hydrogen peroxide by the catalase in 1 gram of the heart of the respective animal

ANIMAL	1	2	3	4	5	AVERAGE
Goose						
Heart	44	42	50	44	52	46
Weight of goose in grams	6000	6500	6700	7000	6120	6464
Pigeon						
Heart	110	100	98	105	109	104
Weight of pigeon in grams	324	315	330	340	328	327
English Sparrow						
Heart	133	111	122	129	113	121
Weight of sparrow in grams	26	30	24	32	28	28

It may be seen in table 1 that the average amount of oxygen liberated by one gram of the goose heart was 46 cc.; by 1 gram of the pigeon heart 104 cc.; and by 1 gram of the English sparrow heart 121 cc. The average weight of the geese was 6464 grams; that of the pigeons, 327 grams; and that of the English sparrows, 28 grams; hence the amount of catalase is greatest per gram of heart in the smallest birds, less in amount in birds of larger size and least in the largest birds.

It may be seen in table 2 that the average amount of oxygen liberated from hydrogen peroxide by 1 gram of the dog heart was 63 cc.; by the rabbit heart, 107 cc. and by the rat heart, 137 cc. The average weight of the dogs was 19,300 grams; that of the rabbits, 2540 grams; and that of the rats, 182 grams; hence the amount of catalase is greatest per gram of heart in the smallest mammal, less in amount in the larger mammal and least in the largest mammal.

Determinations (4) have been made of the catalase content of a great many different kinds of insects. It was found that a single firefly, weight 11 mgm. ground up in sand liberated about 50 cc. of oxygen from 45 cc. of hydrogen peroxide in ten minutes; that is, 1 gram of firefly would liberate about 5000 cc. of oxygen in ten minutes. Other insects such as the honey-bee, bumble-bee, moth, etc., were found to be very rich in catalase, but not so much so as the firefly. It will be noted that 1 gram of firefly contains about one hundred times as much catalase as 1 gram of dog heart.

TABLE 2

After heart, under dog, rabbit and rat, are given the number of cubic centimeters of oxygen liberated in ten minutes from 45 cc. of hydrogen peroxide by the catalase in 1 gram of the heart of the respective animals

ANIMAL	1	2	3	4	5	AVERAGE
Dog						
Heart	50	75	73	53	68	63
Weight of dog in grams	25,000	12,000	16,000	27,500	16,000	19,300
Rabbit						
Heart	76	74	82	91	70	79
Weight of rabbit in grams	2,300	2,600	2,000	2,272	2,640	2,332
Rat						
Heart	133	145	140	115	150	137
Weight of rat in grams	200	210	250	150	100	182

Several observers (5) have pointed out instances in which the Surface Area Law does not hold. Lusk (6) showed that the metabolism of a dog kept in the laboratory decreased very considerably (19 per cent) from what it was when the dog was kept in the country, although the weight of the dog, and hence the surface area, remained constant. Benedict (7) showed that the metabolism of a man during a thirty-one-day fast decreased considerably more than what could be accounted for by the decreased surface area during the fast. He also observed that the metabolism rose during the last few days of the fast although the surface area of the subject was still decreasing. The failure of the law in some instances has been attributed to the inaccuracy in methods for determining the surface area, nature of the integument, variations in amount of body surface exposed, etc., but with more exact methods for determining the surface area it is still found that the law does not hold under many normal as well as pathological conditions, hence Benedict (7) concludes that the loss of heat from the body surface can not be the determining factor and that of the numerous factors affecting metabolism, the internal stimulus to metabolic activity is one of the greatest.

Burge (3) showed that when pigeons accustomed to an active life were confined, the catalase content of the pectoral muscles decreased by 25 to 30 per cent although the weight of the animals remained constant, and that when the animals were given their accustomed amount of exercise the catalase content increased to the normal amount. It is not improbable that the decrease of 19 per cent in the metabolism of Lusk's dog leading an inactive life was due to a decrease in the catalase content of the tissues of the animal. In some unpublished results from this laboratory it was found that the catalase content of the muscles of rats during the first few days of fasting was increased above the normal particularly when the animal was given a small amount of protein. This observation carries the suggestion that the specific dynamic action of proteins may be explained by an increase in catalase, a suggestion which we hope to follow out experimentally.

SUMMARY

Evidence is presented to show that the content of heart-muscle in catalase, per unit of weight, bears an inverse relationship to the size of the animal, or a direct relationship to the intensity of the oxidation processes.

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THE SIGNIFICANCE OF PROTHROMBIN AND OF FREE AND COMBINED THROMBIN IN BLOOD-SERUM

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The fresh serum expressed from the blood clot when added to oxalated plasma or to fibrinogen soon produces coagulation. This thrombic power of serum decreases rapidly, especially at body temperatures, but may be reestablished by various procedures, such as addition of tissue extract or by a transient increase in alkalinity. The nature of these inactive stages of thrombin has been the subject of a number of investigations, the result of which has been considerable difference of opinion in regard to the interpretation of the different procedures of activation. It will be shown as a result of the following experiments that they are capable of a simple explanation which does not involve the assumption of any substance other than those quite generally recognized as coagulation factors.

It was the opinion of Alexander Schmidt (1) that "prothrombin" is only partially activated[2] in the process of coagulation and appears in serum together with thrombin. He believed that prothrombin remains in serum much longer than thrombin and may be activated by the addition of zymoplastic (thromboplastic) substance or by a transient increase of alkalinity, or by both, the alkalinity favoring the action of the zymoplastic substance.

A distinct advance was made by Morawitz (2). He found that there is more than one proferment. He did not believe that the proferment activated by calcium, described by Pekelharing (3) and which we now know as prothrombin, could exist in serum, as blood contains sufficient calcium to activate all the prothrombin, and addition of calcium to serum does not increase its thrombic power. However, he described a proferment distinct from that of Pekelharing which is activated by a change of the H^+ ion concentration of the serum. This he believed was identical with the "prothrombin" of Schmidt. To distinguish the two proferments he called the one activated by calcium α proferment, and the one activated by a change of the H^+ ion concentration β proferment. β proferment does not exist in the circulating blood or oxalated plasma but exists in serum, whether fresh or old.

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[1] This work was started in the physiological laboratory of Johns Hopkins University and most of the experiments were performed at that place.

[2] Schmidt used the word 'Spaltung.' The translation activation is merely the commoner terminology and is not intended to designate the true nature of the process.

In the same year Fuld (4) described the substance in serum which he called metazyme, which is activated by alkali or acid and which he regarded as a more stable phase of thrombin. This substance is to be regarded as identical with the β prothrombin of Morawitz. The latter did not agree that this substance is a more stable phase of thrombin but offered no other explanation. As it seemed most probable that the substance was a modification of thrombin, he suggested the term "metathrombin." For the sake of convenience this term will be used to denote the substance or condition which admits of the development of thrombin when serum is treated with alkali. In a later paper Morawitz (5) revised his view that α prothrombin does not exist in serum as he found it to contain a thrombogen[3] activated by tissue extract in the presence of calcium. That this thrombogen is not identical with metathrombin was evident from the fact that a serum may contain the latter when the former has disappeared. He further presented two other facts which have been confirmed in this investigaiton; serum may be activated by alkali and acid in the absence of calcium but cannot be activated by tissue extract unless calcium is present.

Pekelharing (6) objected to the assumption of the two ferments by Morawitz. It is his belief that serum contains thrombin but no proferment. When serum stands exposed to the air substances arise which oppose the coagulation of fibrinogen and these substances are rendered inactive by acid and alkali. These inhibiting substances are stated not to act on thrombin (7). However, Landsberg (8) came to the conclusion that thrombin is inactivated by adsorption on to the serum proteins. Such an adsorption compound corresponds to the metathrombin of Morawitz. The adsorption takes place in the same manner that trypsin, emulsin and rennin are taken up and inactivated by charcoal, casein or serum albumen.

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[3] Morawitz differentiates this thrombogen from α prothrombin. The α prothrombin was originally defined as a substance activated by calcium. Later when he introduced his theory of a thrombokinase he defined α prothrombin as kinase plus thrombogen without calcium.

Weymouth (9) studied the problem of the activation of serum and came to the conclusion that the substance described as metathrombin is a combination of thrombin with the antithrombin of the serum. The contention of Mellanby (10) and of Collingwood and MacMahon (11) that such a combination is not broken up by the alkali-acid activation is readily disproved. These authors believe that what really happens is a liberation of thrombokinase from the serum proteins on to which it has been adsorbed. If this were the case then the phenomenon would not take place if oxalated serum were activated and tested on oxalated plasma.

METHODS

Only a brief note as to methods and materials used is necessary. Thrombin was prepared by the method described by Howell (12). For the thromboplastic substance occurring in tissue extract cephalin (13) was used. Fibrinogen was prepared by precipitation from oxalated plasma by half saturating with sodium chloride, the precipitate formed being aggregated by the centrifuge, washed with a half saturated solution of sodium chloride and dissolved in a 2 per cent solution of sodium chloride. This procedure was repeated three times and only such preparations were used as did not give a clot in twenty-four hours, when calcified in the presence of cephalin. In some experiments where the action of calcium did not enter oxalated plasma was used. The coagulation time was determined in homoeopathic phials. The coagulation times recorded in this paper, unless otherwise noted, were taken at the time that the phial could be inverted without disturbing the clot.

The method of alkali activation used was practically the same as that used by previous observers. The serum was incubated at 37° for ten minutes with an equal volume of $\frac{N}{10}$ NaOH, then neutralized with $\frac{N}{10}$ HCl using neutral red as indicator. In the later experiments it was found that room temperature was equally satisfactory.

Antithrombin (14) was determined by incubating serum (heated to 60° for ten minutes to remove thrombin and prothrombin) with thrombin for a definite time in the incubator at 37°, then determining the ability of the mixture to coagulate fibrinogen.

EXPERIMENTS

There are two possibilities as to the origin of thrombin in activated serum. It may arise from some alteration of thrombin itself, either by a change in structure of the thrombin on standing or its modification in some way by substances present or developing in serum, or it may arise from prothrombin such as exists in plasma. The former possibility will be first considered.

It has been the general experience of workers with blood that aqueous solutions of isolated thrombin on standing do not undergo inactivation. This fact makes it probable that the inactivation of thrombin in serum is not due to a change in the structure of thrombin. We must turn then to the other alternative, that thrombin is inactivated in serum through the intervention of some substance contained in the serum.

Landsberg considered that the thrombin is adsorbed on to the serum proteins just as rennin may be adsorbed by such a substance as casein. Morawitz (2) tried the action of eggwhite, milk, dextrin and commercial egg albumen on coagulation of fibrinogen by serum and found that none of them caused an appreciable delay. Rettger (15) postulated that if thrombin unites with a protein there must be a marked specificity in this respect as serum soon inactivates thrombin, while but little change takes place when thrombin stands in contact with egg albumen. That there is a specific antithrombin in the blood has gained considerable support from Howell and his coworkers, and Weymouth decided that the metathrombin of Morawitz is a combination of such a substance and thrombin. Therefore in this investigation for comparison with serum a series of determinations of the antithrombin power of various substances was made. Serum was heated to 60° to remove its thrombin and then amounts of one drop were incubated for fifteen minutes at 37° with 2, 3 and 4 drops of thrombin solution. Under exactly duplicate conditions the following substances were incubated with like amounts of the same thrombin solution: (1) Fresh whole milk; (2) fresh egg albumen; (3) peptone ("Witte") 6 per cent solution; (4) potato starch (a thick paste); (5) acacia, 6 per cent solution; (6) gelatine, 6 per cent solution; (7) animal charcoal, 6 per cent suspension; (8) kaolin, 6 per cent suspension.

The thrombin used was of such a strength that two drops clotted ten drops of oxalated plasma in 1 + minutes. When incubated with the above mentioned substances for 15 minutes all the preparations clotted in between 1 and 2 minutes, on the other hand the thrombin incubated with the serum antithrombin was so much inactivated that determination of a definite end point was impossible as is seen in the following data.

ANTITHROMBIN	INCUBATED 15 MIN. WITH THROMBIN	COAGULATION TIME
1 drop	2 drops	Pellicle 2 hours; did not get solid
1 drop	3 drops	Pellicle 75 minutes; gel 4 hours solid 8–20 hours
1 drop	4 drops	Weak gel 20 minutes, solid 8–20 hours

The above experiment shows that the inactivation of thrombin by other substances than serum is negligible when compared with that of serum, in fact it is so small that it could not be measured by the method used. This fact allows of but one conclusion, that the action between serum and thrombin is of a specific nature.

We have seen that thrombin is inactivated by serum, it remains to be shown that a union is formed which answers to the specifications of metathrombin.

It was argued by Morawitz that metathrombin could not be such a combination, as it occurs in old serum from which antithrombin has disappeared. As we now have improved methods (14) of testing for antithrombin this work was repeated. Serum obtained on September 29 was allowed to stand in a stoppered bottle on the laboratory shelf and was tested from time to time for metathrombin and antithrombin. These were found to occur together. November 27 was the last day on which any thrombin could be developed by alkali activation. On this day the antithrombin test was as follows.

THROMBIN	INCUBATED FOR 15 MIN. 37°C WITH	CLOTTED 10 DROPS OF OXALATED PLASMA IN
4 drops	Water 2 drops	8 minutes
4 drops	Serum 2 drops	Jelly clot over night

After this no thrombin could be developed by alkali activation and antithrombin tests showed no appreciable amount of this substance.

A study of the susceptibility of the serum constituents to heat furnished additional evidence of a thrombin-antithrombin combination. Thrombin is destroyed at a lower temperature than antithrombin. It is to be expected therefore that the susceptibility of the combined-thrombin would approximate that of antithrombin in a manner similar to the protection afforded to heat by a substrate towards an enzyme, as peptone to trypsin, Biernacki (16). It is stated by Morawitz (17) and confirmed by Weymouth (9) that thrombin and metathrombin have the same thermolability. On the other hand Rettger (15) found that if a serum be heated for a few minutes at 65° it is inactive when added to a fibrinogen solution but becomes active on treating with alkali and acid.

At a proper selection of temperature and duration of heating it was found that there is a definite difference in susceptibility of thrombin, prothrombin and metathrombin. Serum and plasma were heated side by side in thin walled test tubes in a water bath to the temperature indicated and held there accurately for five minutes. To remove the fibrinogen from the plasma it had been previously heated to 55° and the precipitate removed by the centrifuge. In both plasma and in serum heated to 60° or over no substance remained which yields thrombin on the addition of calcium in the presence of thromboplastic substance. The fate of thrombin, metathrombin, and antithrombin is shown in the following table. In the figures given for antithrombin one drop of serum was heated with the indicated amount of thrombin for 15 minutes at 37°. The thrombin was of such a strength that two drops clotted ten drops of fibrinogen solid in 1 minute.

TABLE 1

SERUM AMOUNT HEATED	COAGULATION TIME		ANTITHROMBIN DETERMINATION		
	Serum 5 drops + fibrinogen 10 drops	Serum 5 drops, activated with alkali + fibrinogen 10 drops	Thrombin 2 drops	Thrombin 3 drops	Thrombin 4 drops
unheated	90 min.	10 min.	flocculent precipitate 60 min.	gel 45 min.	10 min.
60.0°	No clot	20 min.	flocculent precipitate 60 min.	gel 45 min.	10 min.
62.5°	No clot	60 min. good gel 35 min.	Pellicle 60 min.	10 min.	3 min.
65.0°	No clot	gel 60 min. solid over night	gel 3 min.	1 min.	1 min.

It is to be seen that progressive heating to 65° injures metathrombin, but 65° for 5 minutes does not destroy it. At the same time the antithrombin is weakened and but little remains at 65°. Above this temperature both are destroyed. On the other hand all sera heated to 60° or more are inactive, as regards free thrombin, showing a clean cut separation by heat of thrombin and metathrombin, the combined-thrombin being destroyed, not at its own critical temperature in serum but at that of antithrombin. This is good evidence that thrombin is in combination with the antithrombin and controverts the view of Pekelharing that the phenomenon of alkali activation is due to the removal of substances developing in serum which prevent the action of thrombin on fibrinogen but which do not combine the thrombin.

If the line of reasoning followed in this investigation holds, then the effect of alkali on serum is to release the thrombin from the antithrombin, therefore the effect of alkali activation on the antithrombin of serum was studied. Serum was activated and its thrombic and antithrombic power determined. It was then heated to 60° for 10 minutes to remove the free thrombin, after which it was reactivated and a determination made of the thrombin and antithrombin, for comparison with the unactivated serum and the serum activated only once. Care was taken in each case that the determination was made on an amount equivalent to four drops of the original serum. The results are here tabulated.

TABLE 2

	CLOTTED FIBRINOGEN 10 DROPS	AFTER HEATING AT 60° FOR 10 MIN. AND INCUBATING FOR 10 MIN. WITH THROMBIN 2 DROPS—CLOTTED FIBRINOGEN 10 DROPS
Serum 4 drops (unactivated)	55 min.	No clot 21 hours.
Serum 4 drops (activated)	10 min.	75 min.
Serum 4 drops (reactivated)	60 min.	5 min.
Thrombin 2 drops	5 min.	

It becomes apparent from these experiments that in the process of alkali activation, simultaneous with the increase of thrombic power of the serum there is a decrease in its ability to inactivate thrombin. This ability becomes nil with the second activation, a fact which explains the statement made by Morawitz that serum can not be activated a third time; there is no antithrombin remaining in condition to combine thrombin.

The most direct proof of the nature of metathrombin is to be obtained by addition of thrombin to antithrombin.. Experiments of this kind (9) have been tried, but it is obvious that an error is introduced when serum is used. In order to heat the serum sufficiently to remove the metathrombin already present would require a temperature that would destroy the antithrombin. Therefore oxalated plasma which had been heated to 60° to remove the prothrombin was used for antithrombin as it has been repeatedly demonstrated that plasma does not contain metathrombin. It was found that small amounts of plasma could inactivate very large amounts of thrombin in a few hours, therefore the plasma was added directly to the watch crystals containing the dried thrombin. The presence of thrombin after alkali activation is not sufficient proof of activation, the free thrombin already present is injured but by no means destroyed in the process. The criterion of a thrombin-antithrombin combination was a plasma which had but slight thrombic activity but which developed thrombin after treatment with alkali and acid. Such a preparation can be obtained with great regularity if the plasma is incubated for the proper time with sufficient thrombin.

The data of two of the most successful experiments are presented as follows:

Twenty drops of plasma were added to a watch crystal in which 5 cc. of a very active thrombin solution had been dried. The mixture was allowed to stand for 24 hours at 30 to 35°, at the end of which time it reacted as follows:

A

Thrombin-plasma mixture, 6 drops + oxalated plasma 10 drops, no clot in 24 hours.

Thrombin-plasma mixture, 6 drops (activated) + oxalated plasma 10 drops, clot in 6 minutes.

Cephalin had no effect on this combination.

In an experiment similar to the above in which different preparations of the constituents were used the following results were obtained:

B

Thrombin-plasma mixture, 5 drops + fibrinogen 10 drops, no clot in 10 hours.

Thrombin-plasma mixture, 5 drops (activated) + fibrinogen 10 drops, clot in 1 hour, gel in 30 minutes.

From the foregoing experiments we may conclude that in serum there exists a combination of thrombin and antithrombin which has the thermolability of the latter, not of the former, which does not yield thrombin on the addition of cephalin but is disrupted by alkali with a liberation of thrombin attended by such an alteration of antithrombin that it becomes inactive.

In addition to the combined-thrombin existing in serum there is also evidence of the presence of prothrombin as was believed by Schmidt and Morawitz. If a small amount of cephalin is added to fresh serum it becomes very active. If the serum is first oxalated no activation occurs. That this is not due to the excess of the oxalate can be shown as no activation occurs after removal of the excess of oxalate by dialysis. That oxalate produces no injury to the prothrombin follows from the observation that normal activation of such a serum by cephalin occurs if it is recalcified. The only substance known in the coagulation system that yields thrombin in the presence of calcium and tissue extract is prothrombin. The thrombin-antithrombin union when once formed has been shown not to be affected by thromboplastic substance as serum heated to 60° for 10 minutes does not develop thrombin on the addition of cephalin and the latter does not split the union between plasma heated to 60° and thrombin.

The time at which the thrombin is combined by the antithrombin is of interest. No combined-thrombin exists in oxalated plasma but it is present in apparently full amount in the fresh serum. The following experiment was devised to determine the time of its origin and the relationship of the amount of tissue extract to the amount of the different stages of thrombin in serum. In order to get a plasma low in tissue-extract content a cat was bled from the carotid through a greased cannula into oxalate. This was centrifuged at once at high speed for over an hour to obtain a very clear plasma. Such a plasma was heated to about 55°C., at which point the fibrinogen is coagulated but the other coagulation factors are undisturbed. The coagulum was removed by means of the centrifuge. If a plasma so prepared is calcified the process of thrombin formation is initiated without the removal of any of the thrombin by fibrinogen, furthermore it is not necessary to wait till after clot formation to study the serum. The plasma was calcified according to an optimum previously determined on the unheated oxalated plasma.

Two series were run simultaneously: Preparation I was low in tissue-extract content (i.e., contained only the small amount unavoidably present in the oxalated plasma); Preparation II was prepared with an excess of cephalin. Thrombin determinations were made on these after oxalating to prevent further conversion of prothrombin. For prothrombin determinations, to Preparation I was added cephalin. Preparation II already having an excess of cephalin was untreated. The coagulation times observed in the prothrombin determinations therefore represent the effect of the thrombin already present plus the effect of that formed from prothrombin after preparation of the coagulating mixture. Combined-thrombin was determined by the alkali activation of a portion that had been heated to 60° for 5 minutes to remove the thrombin and prothrombin present. The slight injury unfortunately occurring in this amount of heating does not seriously interfere with the results. It necessitates, however, that the heating of each specimen should be exactly the same. It is obvious, in experiments of this kind where tissue extract and calcium are both present, that the tests, to be of value, had to be made on a fibrinogen preparation absolutely free from prothrombin, that is without a trace of clotting after a 24-hour contact with calcium and cephalin.

Determinations were made at periods varying from 1 minute to 24 hours after calcifying. The results obtained are plotted in figures 1 and 2.

As the observations lasted 24 hours and as the maximum changes occurred in the first few minutes it was necessary to plot the points on a system of logarithmic coördinates to get the results within the curve. The axis of abscissae represents the time in hours after calcification of the plasma, the axis of ordinates represents the amount of thrombin as indicated by the reciprocal of the coagulation time expressed in hours. It should be borne in mind that the coagulation time is an index to the amount of thrombin only up to a certain maximum amount above which the coagulation time can not be shortened by an increase of thrombin present (15). Therefore in the prothrombin determinations in Preparation I there was during the whole 24 hours at least sufficient prothrombin to give coagulation in the minimum time, one minute or less, except in the last test where the coagulation time was 2 minutes. There must have been a diminution of the prothrombin as thrombin was formed but this never reached a point below the maximum that could be determined. In contrast with this in Preparation II where there was an excess of tissue extract, the prothrombin was converted to thrombin at the very start of the experiment. The prothrombin content of the serum must very soon fall to zero. It is seen that the prothrombin curve follows the thrombin curve very closely. At most only the difference between the two curves can represent prothrombin and this difference must be still further discounted, as in the preparation on which the thrombin was determined a precipitate of calcium oxalate had been formed which would carry down some of the thrombin.

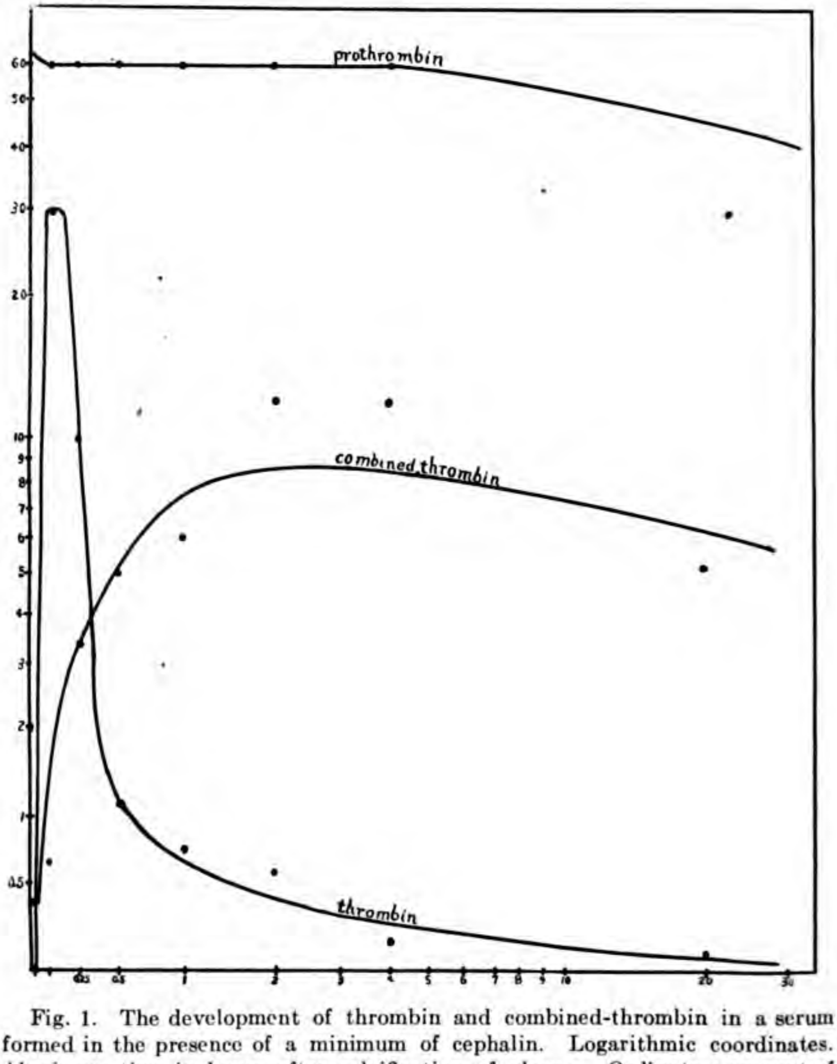

Fig. 1. The development of thrombin and combined-thrombin in a serum formed in the presence of a minimum of cephalin. Logarithmic coordinates. Abscissae: time in hours after calcification of plasma. Ordinates: amount of thrombin as indicated by the reciprocal of the coagulation time expressed in hours.

The rapidity of conversion of prothrombin explains the great variability of sera in relation to tissue extract. In the early part of the work the fact that cephalin would not increase the thrombic power of many sera was a perplexing phenomenon especially as it was not known that cephalin would not liberate combined-thrombin. As the activation of a serum by tissue extract depends upon its prothrombin con-

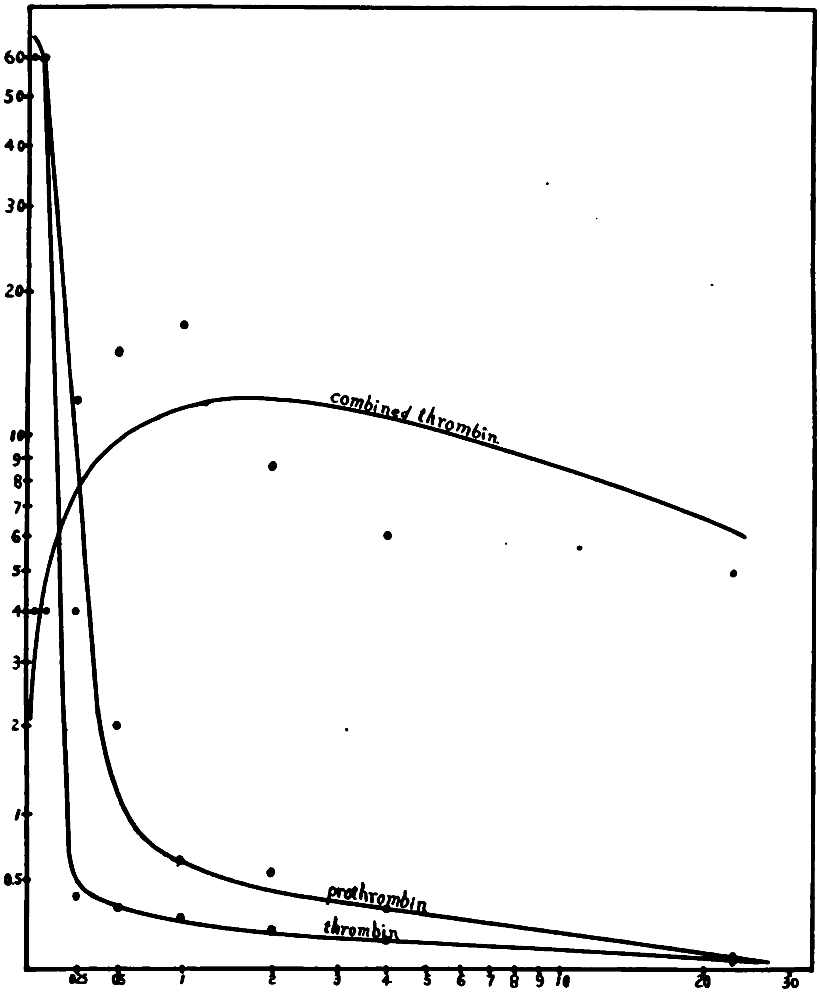

Fig. 2. The development of thrombin and combined-thrombin in a serum formed in the presence of an excess of cephalin.

tent, and this in turn depends upon the amount of tissue extract already present, it can be seen that conversion of prothrombin is so complete when enough of the latter is present during clotting that no more remains in the serum to be liberated by thromboplastic substance.

The thrombin curves in the two cases show two points in common. There is an early rapid rise in amount followed by a rapid fall. The rise is best seen in Preparation I, no thrombin was demonstrable in the one minute sample, but it reached its maximum after 5 minutes. In Preparation II the rise was so rapid that in the one-minute specimen coagulation was in the minimum time. In both cases the disappearance of the thrombin was strikingly rapid. This rapid disappearance of thrombin which is undoubtedly also taking place even while the total amount is rising is attended by an increase in the amount of combined-thrombin comparably rapid.[4] This explains the observation of the older observers that metathrombin is present in maximum amounts in fresh serum. By the time the serum could be obtained from the clot sufficient thrombin had combined to give the maximum reaction. There is no evidence whatever in the curves that thrombin is constantly being combined; the amount of thrombin that the amount of tissue extract is capable of producing is formed rapidly, then quickly disappears so that the maximum of the combined-thrombin curve is reached very early, after which the amount as far as it can be determined remains nearly stationary, at least there is no further rise. The slight fall in the curves is to be explained in part by the deterioration of the fibrinogen which takes place during the period of the experiment. The decrease in combined-thrombin in serum must be very gradual as it is still present in large amounts after several weeks. The apparent variation in the points on the curve is due to the method of plotting. The maximum variation in the coagulation times observed after the curve reaches its maximum, is only 8 minutes and this is greatly influenced by slight variations in the H^+ ion concentration of the end point after neutralization.

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[4] The slight injury caused by the heating to 60° prevents the rise of the combined-thrombin curve to the height it would attain if determinations were made on unheated preparations. On the other hand a curve determined under the latter conditions would be very inaccurate in parts of the curve showing large amounts of free thrombin.

DISCUSSION

The foregoing facts have a direct bearing on the procedure of the coagulation process. It is a well known fact that large amounts of thrombin can be injected into the circulating blood without the occurrence of thrombosis. In figure 1 is written the story of a comparable situation. The small amount of thromboplastic substance present in the plasma was capable of forming rapidly a definite amount of thrombin, but this thrombin disappeared about equally as rapidly, the rise of the combined-thrombin curve showing the manner of its removal. As thrombin can not be liberated from the blood antithrombin by any agency known to be present in normal blood the small amounts of thrombin that may arise as a result of thromboplastic substance becoming available are permanently removed from the possibility of activity. In the blood stream the removal would be even more rapid. The experiments were performed at room temperature whereas at body temperature the activity of antithrombin is very much greater, Howell (18). In normal coagulation the thrombin when formed has two possible fates. It may be combined by antithrombin or by fibrinogen. The division coefficient between these two would be a valuable bit of information. It is to be expected that the presence of the fibrinogen would greatly modify the course of the combination of thrombin by antithrombin. We may cite for analogy the relatively greater inhibition of rennin by serum-albumen, Hedin (19), when the rennin has been in contact with the serum-albumen before the addition of the milk. It would seem that such a combination of thrombin by antithrombin would be very detrimental to the process of coagulation but during conditions allowing clotting the coefficient of division would be modified in favor of the fibrinogen combination by the decrease of temperature and by the presence of thromboplastic substance. There is an undoubted delay in the thrombin-antithrombin union in the presence of cephalin as is shown very clearly in Howell's experiments (13). This delay is not sufficient, however, to prevent a rapid development of combined-thrombin when a great deal of thrombin is present (vid. fig. 2), or to maintain thrombin free in the serum for any length of time.

The properties of the thrombin-antithrombin combination have a relationship to the nature and condition of prothrombin. The resistance of the combination to cephalin precludes the possibility that prothrombin is a thrombin held in an inactive form in the circulatory plasma, at least as an antithrombin combination. According to the theory of coagulation formulated by Howell, prothrombin is protected from calcium activation by antithrombin. Such a prothrombin-antithrombin complex would seem to be different in nature from the thrombin-antithrombin combination as it is broken up by thromboplastic substance and the prothrombin retains its own critical temperature, not being protected by antithrombin.

The facts known at present concerning the nature of the combination in which thrombin is held does not allow of its definite classification. If an adsorption it would be one in which the substance adsorbed is fixed in such a way that the process is not reversible. Hedin (20) has described such a process for the inactivation of enzymes. The inactivation of trypsin or rennin by serum albumen or egg white differs in many ways from ordinary adsorption. The enzyme may be completely removed if sufficient of the inactivating agent is present. The amount of enzyme taken up increases with the temperature and it is independent of the amount of water present, the adsorption compound is therefore not broken down by the addition of water. The fact that a combination of the enzyme with charcoal occurs which has the same properties has led Hedin to class the process with the adsorptions. If the serum albumen or egg white is treated with acid, then neutralized, it loses its power to combine an enzyme. The analogy of the Hedin adsorption with thrombin inactivation led Landsberg to assume that thrombin is adsorbed by serum albumen. In many ways the analogy holds. However, as has been pointed out, the inactivation of thrombin by serum is all out of proportion to its inactivation by charcoal or eggwhite and one would be forced to assume that there is a marked specificity in the reaction whether this be due to a favorable chemical configuration of the surface of the antithrombic phase or a more rapid or complete fixation of the substance adsorbed. The Hedin adsorption is partially reversible. When the substrate of the enzyme is brought into contact with the adsorbed enzyme a part of the substrate is adsorbed and forces out a portion of the enzyme, which acts upon the unadsorbed substrate. In the case of the thrombin-antithrombin combination, fibrinogen does not produce this effect as a serum heated to 60° when added to fibrinogen does not produce a clot although it may be proved by alkali activation to be rich in combined-thrombin. There is at present, in so far as is known to the author, no evidence that thrombin-antithrombin reaction is reversible; processes which break up the combination also injure or destroy the antithrombin.

SUMMARY

1. There is only one kind of thrombin in serum. This may be formed from prothrombin by the action of thromboplastic substance and calcium or may be liberated from a combination with antithrombin by alkali activation according to the method of Schmidt.

2. The inactive stage from which thrombin is liberted by alkali activation is believed to be a thrombin-antithrombin combination for the following reasons:

(a) The inactivation of thrombin by non-specific colloids, charcoal and kaolin is negligible compared with the inactivating action of plasma.

(b) Combined-thrombin and antithrombin disappear at the same time from serum allowed to stand.

(c) Combined-thrombin is destroyed by heat not at the critical temperature of thrombin but at that of antithrombin.

(d) In the process of alkali activation the antithrombin is destroyed or so altered that it loses its ability to combine thrombin.

(e) When thrombin is incubated with antithrombin (i.e. plasma heated to 60°) a combination is found which does not clot fibrinogen until broken up by the alkali activation method.

3. Thrombin is not liberated from combination with antithrombin by the action of thromboplastic substance.

4. The amount of prothrombin in serum varies inversely as the amount of tissue extract present at time of clotting.

5. The amount of thrombin formed in the presence of a given amount of thromboplastic substance is, apparently, definite in amount; it forms rapidly and is combined rapidly, the system then returning to equilibrium. This rapid inactivation is a potent factor in the maintenance of the normal fluidity of the blood. Plasma is very intolerant of free thrombin.

It is a great pleasure to me to thank Dr. Howell for suggesting this problem and for the kindly suggestions received during its solution.

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THE POSTURAL ACTIVITY OF THE ABDOMINAL MUSCLES OF THE CAT

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Sherrington (1) has recently called attention to the general postural activity of muscle and nerve in animals. In a study of the neuro-muscular respiratory mechanism we had occasion to investigate the rôle of the abdominal muscles in the respiratory movements. In the course of these experiments we were impressed with the great variations in length of the rectus abdominis muscle, and decided to look into the cause of these variations a little more closely.

Our experiments were done on cats under continuous ether anesthesia and without decerebration. Respiratory tracings were taken from the thorax and the abdomen by means of a Crile stethograph (2), connected to a Verdin recording tambour. The rectus abdominis muscle was exposed by dissecting away the lower medial portion of the pectoral muscles of one side and exposing the attachment of the rectus abdominis to the ribs. A thread attached to the upper end of the rectus abdominis, after severing its attachments to the ribs and dissecting it down to the beginning of the transversalis abdominis, was attached to a muscle lever whose writing point was directly beneath that of the recording tambours. The movements of the lever indicated the variations in the length of the muscle, a fall of the lever indicating a relaxation. Some movements of the muscle lever occurred with each respiration, but these ordinarily were not very great. In dyspnoea the movements of the rectus abdominis might increase very greatly. This phase of its activity will, however, be discussed in a separate communication. We wish, at this time, simply to call attention to its active changes in length with changes in the intra-abdominal pressure.

Our first observations were made by distending the stomach with fluid by means of a stomach tube inserted through the esophagus. To prevent regurgitation when the pressure of the fluid became more than a few centimeters of salt solution we found it necessary to pass a ligature about the esophagus in the neck. We discovered that it was impossible, however, to reach the limits of distension of the abdominal wall by injecting fluid into the stomach. We therefore injected an $\frac{M}{8}$ solution of sodium chloride directly into the peritoneal cavity by means of a burette and a hypodermic needle. The injection of the fluid was usually continued until no more would run in under a pressure of a column of fluid 80 to 100 cm. high.

When the central nervous system and the afferent nerve roots are intact, the introduction of 100 cc. of fluid into the stomach or the peritoneal cavity is attended by a slight transient increase in the abdominal girth, as shown by the tracing of the abdominal respiratory movements, and a marked increase in the length of the rectus abdominis muscle. The introduction of small amounts of fluid, 10 to 25 cc., may be sufficient to bring about a noticeable change in the length of the rectus abdominis and a small increase in the girth of the abdomen. Subsequent injections of fluid in quantities of 100 cc. are, of course, attended by an increase in the abdominal girth and by a lengthening of the rectus abdominis muscle until the limit of distensibility of the muscle is reached. The pressure under which the first injections were made was not more than 5 or 10 cm. of salt solution, and fluid will continue to enter the peritoneal cavity at these low pressures of injection until one approaches the limits of extensibility of the abdominal wall, when the pressure required for injection rather suddenly rises. When the pressure of injection becomes relatively high, practically every trace of diaphragmatic respiration ceases, as shown by the minimal excursion of the writing point of the tambour connected with the abdominal stethograph. The costal respiration increases as the abdominal respiration becomes more and more feeble. We cite below a protocol of one typical experiment, in which the fluid was injected directly into the stomach (A) when the central nervous system was intact and (B) after section of the dorsal roots of certain spinal nerves.

Cat. June 14, 1916

Ether. Tracheotomy. Laminectomy before any records were taken, but the dorsal nerve roots were not divided at first.

Fluid introduced into stomach through a stomach tube. Approximately $\frac{1}{8}$ molecular solution of sodium chloride was used.

12.40 a.m. 100 cc. fluid injected. Left rectus abdominis muscle prepared.
12.44 a.m. 100 cc. fluid injected.
12.45 a.m. 100 cc. fluid injected. See figure 1.

12.51 a.m. 100 cc. fluid injected.
12.54 a.m. 100 cc. fluid injected.
12.56 a.m. 100 cc. fluid injected.
12.58 a.m. 100 cc. fluid injected.
1.00 p.m. 84 cc. fluid injected.

NOTE. The fluid was observed to flow into the stomach during inspiration, and to remain stationary in the burette or to flow in more slowly at other times. The pressure at which the flow into the stomach ceased was 37 cm.

As we desired to test out the reactions of the same animal after section of the dorsal nerve roots, the end of the stomach tube was depressed and the fluid allowed to flow out of the stomach. In this way about 470 cc. of the 784 cc. injected was recovered in the next twenty-five minutes. The flow of the fluid toward the end was faster during expiration than at other times. The dorsal roots of the spinal nerves were then exposed from the second thoracic to the second lumbar segment and divided. The animal was then turned on its back, the apparatus readjusted and the introduction of fluid into the stomach begun.

1.55 p.m. 100 cc. fluid injected. The respiration was now almost wholly diaphragmatic, the ribs moving only a little.
1.57 p.m. 100 cc. fluid injected.
1.58 p.m. 100 cc. fluid injected.
1.59 p.m. 100 cc. fluid injected.
2.01 p.m. 100 cc. fluid injected.
2.04 p.m. 78 cc. fluid injected. The pressure at which no more fluid entered the stomach was 42 cm. The respiration changed rather suddenly and became predominantly costal, the movements of the diaphragm decreasing greatly. The lever attached to the rectus abdominis now rose. See figure 2.
2.06 p.m. 50 cc. fluid injected. Pressure 61 cm.
2.07 p.m. 30 cc. fluid injected. Pressure 72 cm.
2.09 p.m. 17 cc. fluid injected. Pressure 80 cm.

Fluid allowed to escape from stomach by mouth. 345 cc. of the 675 cc. escaped.

At autopsy the stomach and intestines were found to contain saline, the intestines being very full.

To show that the lengthening of the rectus abdominis with increase in the intra-abdominal pressure is dependent upon centripetal impulses over the afferent nerves from the abdominal region, we repeated the experiments after exposing the spinal cord and dividing the dorsal roots through which the afferent nerves from the abdominal muscles enter the spinal cord. These nerves, particularly those supplying the rectus abdominis, in the cat enter through the lower thoracic roots.

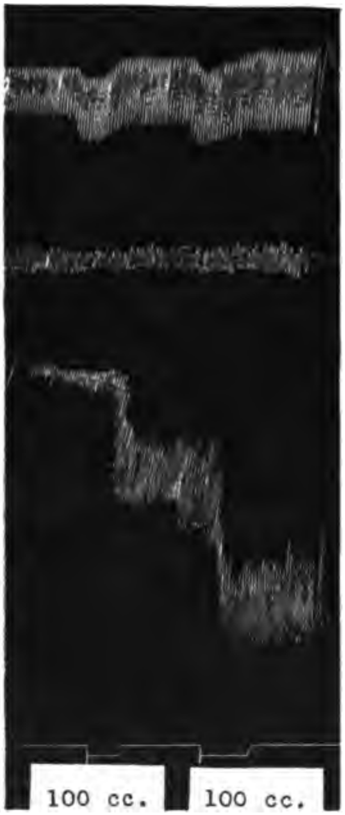

Fig. 1.

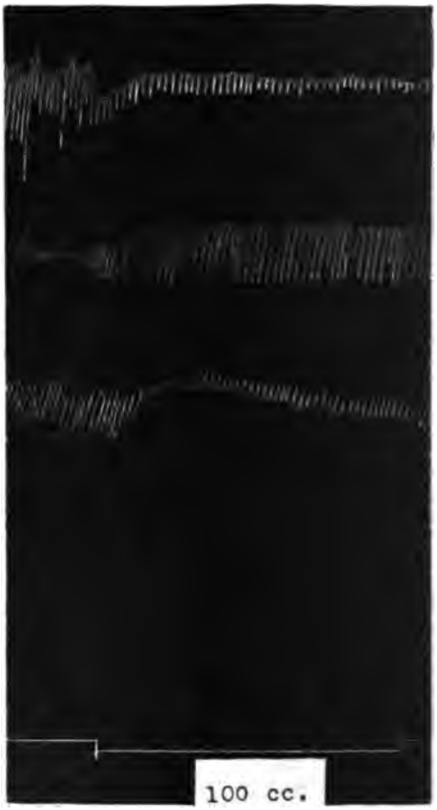

Fig. 2.

Fig. 1. The upper tracing is the abdominal respiration, the second from the top the thoracic respiration, and the lower tracing the rectus abdominis muscle. Two separate amounts of fluid of 100 cc. each were introduced into the stomach during the period of time indicated by the depression of the signal (lower line), with an interval of about one minute between injections. The depression in the upper tracing indicates an increase in the abdominal girth. The abrupt drops in the lower tracing indicate a lengthening of the rectus abdominis.

Fig. 2. The order of the tracings is the same as in figure 1. The dorsal roots of the spinal nerves had been divided. 100 cc. of the fluid was introduced into the stomach during the period indicated by the depression of the signal, the slower inflow resulting from the fact that the stomach wall was approaching its limit of distention. The respiration changed rather suddenly from a predominant abdominal type to a costal type, as indicated by the relative magnitudes of excursion of the writing points of the two tambours. The rise in the curve of the rectus abdominis shows that the muscle is no longer lengthening, or at least not lengthening sufficiently to keep pace with the increase in stomach contents.

We exposed the greater part of the thoracic and the upper part of the lumbar region of the spinal cord, cutting all the dorsal roots on either side. The skin was drawn together with stitches, the animal turned on its back, and all the adjustments of stethographs and writing lever made as before. When the fluid was introduced directly into the stomach, most of it could be recovered by lowering the end of the tube through which the fluid was introduced, and the same animal could be used to show the deportment of the rectus abdominis before and after division of the dorsal nerve roots. We were not so successful in recovering the fluid from the peritoneal cavity, and had to confine ourselves to one experiment on each animal when the fluid was introduced through a hypodermic needle in the abdominal wall.

There may, at first, be some slight passive changes in the length of the rectus abdominis muscle after division of the dorsal roots of the spinal nerves and there are, of course, changes in the abdominal girth following each introduction of fluid into the peritoneal cavity, but the changes in the rectus abdominis at this early period in the experiment are neither consistent nor typical and we attribute them, in part, to the passive mechanical changes in the position of the lower ribs through distension in the abdominal cavity. These passive changes constitute a source of error in both sets of experiments, but if these changes were the only agencies involved in the lengthening of the rectus abdominis, the curves taken before section of the dorsal roots of the spinal nerves should be identical with those taken after section. They are not, however, identical as will be seen on referring to figures 1 and 2. The mere fact that the curve shown in figure 2 was made when the pressure in the stomach was significantly higher—42 cm. of salt solution—than when the curve shown in figure 1 was recorded is not the reason for the difference in the curves. When the dorsal roots are intact, the rectus abdominis continues to lengthen as long as any fluid enters the stomach. When the dorsal roots are divided, the curve of the rectus abdominis is irregular, and for the greater part of the experiment, there may be no movements of the muscle lever other than those dependent upon respiration. When the intra-abdominal pressure begins to rise, the curve indicates by a rise that the muscle is no longer lengthening, or at least, not lengthening sufficiently to compensate for the rise in intra-abdominal pressure.

The pressure at which the flow of fluid into the stomach begins to slow down or stop is not significantly different after section of the dorsal roots from the corresponding pressure observed before their division.

Before dividing the dorsal roots, the flow of fluid into the stomach stopped when the pressure fell to 37 cm. of salt solution. After division of the dorsal roots, the flow of fluid into the stomach slowed down and practically ceased when the height of the column fell to 42 cm. But the maximal distention of the stomach was not reached. By increasing the pressure of injection until the height of the column of fluid reached 80 cm., 97 cc. more fluid entered the stomach. One may infer that the dorsal roots of the thoracic region of the spinal cord have little effect on the pressure at which the walls of the stomach begin to offer resistance to the further entrance of fluid. After a second withdrawal of the fluid from the stomach and division of both vagi in the neck, resistance to a third injection of fluid was first manifested when the pressure was that of a column of salt solution 60 cm. high. This difference in pressures may have been due to the undue distention of the stomach by the previous high pressure of 80 cm., and not to the vagotomy. The division of the vagi was not repeated in any other experiment.

Water was the first fluid which we used for injecting into the stomach, but diuresis was so free that it was necessary to close the urethral orifice with a clamp in order to get the desired degree of distention of the abdominal walls. We next tried a concentrated solution of sodium chloride, on the theory that smaller amounts of a fluid whose concentration of inorganic salts would be high would be separated from the blood by the kidneys, than when water was injected into the stomach. The use of the concentrated salt solution was, however, open to some objections. At autopsy, the mucosa of the stomach and intestines was much inflamed. The presence of the concentrated sodium chloride in the intestine also induced free peristalsis. The $\frac{M}{8}$ solution of sodium chloride, with closure of the anal and urethral openings was the most satisfactory procedure.

When fluid is injected directly into the peritoneal cavity, the curve of elongation of the rectus abdominis muscle is similar in all respects to that obtained when fluid is injected into the stomach, after section of the dorsal roots of the thoracic nerves as well as when these nerve roots are intact. When the dorsal roots are intact, the fluid may enter the abdominal cavity most rapidly during expiration after the cavity begins to fill up. The rectus abdominis muscle continues to lengthen slightly, the writing point of the lever never rising, until no more fluid enters at anything like normal pressure, the pressure of injection in one experiment rising until the column of fluid was 83 cm.

The limits of extensibility of the abdominal muscles, as inferred from the pressure at which no more fluid enters, are not greatly different after section of the dorsal roots. In one experiment in which the dorsal roots of the spinal nerves were intact, the resistance to the entrance of more fluid into the peritoneal cavity appeared when the height of the column of fluid fell to 54 cm. That is, the pressure of a column of salt solution more than 54 cm. high was necessary to force any more fluid into the abdomen. In another experiment, after section of the dorsal roots, the first resistance to the entrance of more fluid was encountered when the height of the column of fluid fell to 45 cm. This agrees fairly closely with the pressure—36 cm. to 42 cm.—at which resistance to the further entrance of fluid was manifested by the stomach. Neither the abdominal wall nor the stomach withstood a pressure of much more than 80 cm. of fluid without slowly yielding, 80 cm. and 83 cm. being typical measurements. We may remark in passing that it is the muscular wall of the abdomen which limits the amount of fluid which can be introduced, and not the skin. In one animal in which the needle gradually slipped from the peritoneal cavity out through the muscle wall into the subcutaneous tissue, during the process of distention of the abdominal cavity, it was found that a relatively enormous volume of salt solution could be introduced and the posterior part of the animal assumed proportions approximating those of a football. Three liters of salt solution were in this way introduced into a young cat which weighed less than 2 kgm. before the fluid was introduced (fig. 3). How much more fluid could have been introduced we do not know. At autopsy the subcutaneous tissues resembled a mass of rather thin imperfectly hardened gelatine.

A point of some interest in connection with the mechanism of regulation of the respiratory movements came out in these experiments. Whereas, normally the thorax participates actively in the respiratory movements of the cat, it was noted that costal respiration ceased after section of the dorsal roots of the thoracic nerves. This cessation was not, however, due to any permanent paralysis of the intercostal muscles or to any anatomical injury to the ventral nerve roots. As the filling of the abdominal cavity proceeded the costal respiration was gradually resumed and at the close of the experiment was the only type of respiratory movement which was present. We may mention, also, that when the dorsal roots of the cervical nerves are divided, leaving the thoracic roots intact, it is the diaphragm which ceases to contract, while costal respiration continues uninterrupted.

We do not know the central mechanism for this adjustment of the length of the abdominal muscles to the changes in intra-abdominal pressure. Evidence has been adduced (3) that some degree of reflex tonus of the skeletal muscles of the cat persists during all phases of

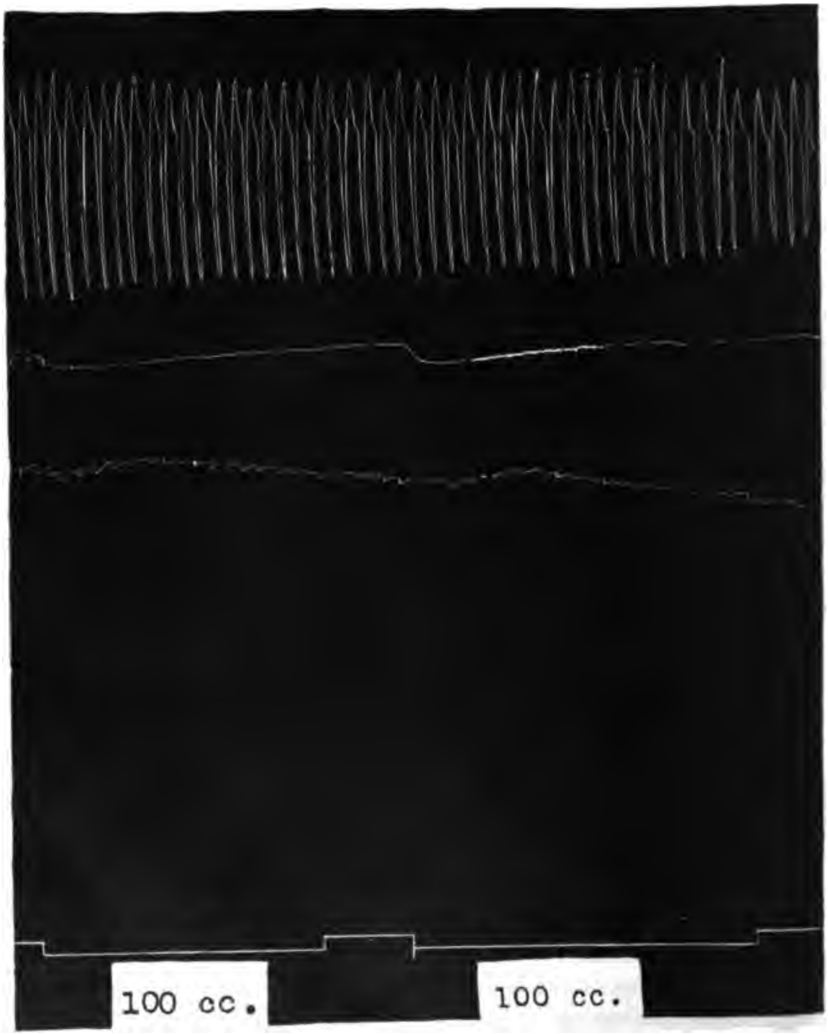

Fig. 3. Upper tracing thoracic respiration, middle abdominal respiration and lower, rectus abdominis. At the time of injection of fluid into the subcutaneous tissues, there is an increase in the abdominal girth, as shown by the depression in the middle tracing, and at first, a shortening of the rectus abdominis succeeded by a slight lengthening.

spinal shock, and that a part of this mechanism must, therefore, be purely spinal. But a purely spinal mechanism does not seem sufficient for all the adjustments which occur. The change from diaphragmatic to costal respiration, or the fading out of the diaphragmatic contraction and the concomitant increase in the depth of the costal respiration almost certainly involve a central mechanism as high up as the medulla oblongata. We are inclined to regard these changes in length of the rectus, and probably of the other abdominal muscles, as part of the respiratory movements, and to be under the control of the respiratory nervous mechanism.

The abdominal muscles may be regarded, in one sense, as antigravity muscles, since they sustain a fairly constant weight of viscera whenever the animal is in a normal posture for locomotion. The question arises whether their postural changes are dependent in any degree upon the cerebellum. We hope to present further data on this and other questions concerning the central mechanism.

We believe that the experiments show that the regulation of the length of the abdominal muscles corresponding to the increase in volume of the contents of the abdominal cavity is a reflex process dependent upon afferent impulses, and that it falls into line with the other known instances of postural activity of muscle and nerve. We may recall here Langley's (4) view that there is a receptive substance in muscle capable of causing a slow contraction, as well as a receptive substance for a quick contraction, and Lingle's (5) observations on the histological appearance of striated muscles. In whatever phase the excised muscle was fixed, Lingle noticed that there were some nuclei which indicated by their shape that the cells to which they belonged were partially or wholly contracted, while other nuclei in adjacent fibers might indicate an almost complete lack of such contraction. It seems probable that the abdominal muscles, which normally undergo considerable changes in length during the life of the individual, must, at all times, have some fibers in a state of contraction, while others are partly or wholly relaxed. Variation in the number of the contracted fibers might be expected to correspond to the degree of passive extension of the muscles.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XL. The Ammonia of the Gastric Juice

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A review of the literature on the chemistry of the gastric juice shows that the findings as to the amount of ammonia and the views as to the origin and significance of this ammonia vary greatly. This discrepancy in findings is no doubt due partly to the difference in character of material analyzed and partly to the different methods of analysis used. The importance of the analytical method which is used is clearly shown by Folin (9) in his work on the ammonia content of the portal blood. A brief summary of some of the work on gastric juice ammonia follows:

OBSERVER	DATE	METHOD OF ANALYSIS	MATERIAL USED	RESULTS
Bidder and Schmitt (2)	1852		Dog with gastric fistula	Trace of ammonia found
Bidder and Schmitt (2)	1852		Man	No trace found
Leo (22)	1892	Schlösing	Man; Ewald meal	Trace of NH_3 in health. More in uremic cases
Rosenheim (30)	1892	Schlösing	Man	Trace of NH_3 as NH_4Cl. Comes from gastric secretion or from decomposition of albumen.
Martius and Lüttecke (24)	1892		Man	NH_3 found only as NH_4Cl in cases of uremia.
Ewald (7)	1892		Man	NH_3 found in 50 per cent of cases examined

OBSERVER	DATE	METHOD OF ANALYSIS	MATERIAL USED	RESULTS
Strauss (36)	1893	Strauss	Man; gastric fistula and gastric disorders	NH_3 in 80 per cent of cases examined
Sticker (35)	1896	Schlösing and Nessler	Man; test meal	NH_3 in gastric juice comes from saliva or from decomposed albumen in stomach disorders
Salaskin (31)	1898	Nencki-Zaleski	Dog; stomach contents	High NH_3 content
Nencki-Pavlov-Zaleski (26)	1898	Nencki-Zaleski	Dog; stomach contents	High NH_3 content
Horodynski-Salaskin-Zaleski (17)	1898	Nencki-Zaleski	Dog; stomach contents	High NH_3 content
Reissner (27)	1903		Dog with Pavlov pouch	NH_3 when found comes from saliva or from decomposed albumen
Sommerfeld (34)	1905		Man with gastric fistula	No trace of NH_3
Rosemann (29)	1907	Schlösing	Dog; esophageal fistula	NH_3 present comes from secretions of stomach
Zunz (40)	1910		Man after test meals	NH_3 present; more abundant in malignancy.
Wolf and Junghaus (39)	1911		Ewald meal	Soluble protein found increased in cases of malignancy
Carlson (3)	1915	Folin-Nessler	Dog; Pavlov pouch	NH_3 in all cases
Carlson (3)	1915	Folin-Nessler	Man: gastric fistula	NH_3 in all cases
Carlson (3)	1915	Folin-Nessler	Man; Rehfus tube	NH_3 in all cases

In an effort to clear up some of the points of difference brought out by this comparison of data and to determine more accurately the factors which influence the amount of gastric juice ammonia, this experimental work was carried out, at the suggestion of Dr. Carlson, on normal and pathological men and dogs. The work is an attempt to throw further light on the following questions:

1. Is ammonia normally excreted from the blood by the gastric mucosa?
2. Is it produced in part by deaminization of amino-acids in the gastric mucosa?
3. Is it a necessary by-product of the normal secretion processes of the gastric mucosa?
4. Is it due to the action of enzymes or the HCl on the gastric juice proteins or on the mucous surface?
5. Is it due to action of bacteria in the stomach?
6. What is its pathological significance?

The ammonia in the pure gastric juice of normal dogs

DOG NO.	MATERIAL	NUMBER OF OBSERVATIONS	NH_3 IN MGM. PER 100 CC. GASTRIC JUICE			AVERAGE ACIDITY	
			Maximum	Minimum	Average	Free	Total
1	1 hour before	13	5.0	0.5	1.7	0.000	0.0093
	1 hour after	14	8.0	0.5	2.1	0.0996	0.1708
	Second hour after	6	6.0	0.5	2.6	0.2598	0.2910
2	1 hour before	6	8.0	2.0	5.0	0.0045	0.0205
	1 hour after	7	5.0	3.0	3.7	0.1029	0.1466
	Second hour after	4	6.0	2.5	4.0	0.2881	0.2903
3	1 hour before	3	5.0	3.5	4.1	0.1029	0.1993
	1 hour after	3	4.0	2.5	3.3	0.2685	0.3054
	Second hour after	2	2.5	2.5	2.5	0.2679	0.3965
4	1 hour after	3	3.5	1.5	2.8	0.1845	0.2393
5	1 hour after	1	2.5	2.5	2.5	0.0729	0.1641
6	1 hour before	2	6.0	2.0	4.0		
7	†1 hour after injection	2	2.5	2.5	2.5	0.2361	0.3085
8	1 hour before	2	2.0	1.5	1.75	0.1995	0.2989
	1 hour after	2	3.0	2.0	2.5	0.2644	0.3209
	2 hours after	2	4.0	2.5	3.25	0.2829	0.3554
	*1 hour before	3	2.0	1.5	1.8	0.000	0.0392
	1 hour after	3	3.5	0.5	2.8	0.1185	0.1905
	2 hours after	3	6.0	2.0	4.0	0.3664	0.4119
9	1 hour before	4	6.0	2.0	4.4	0.1130	0.1840
	1 hour after	4	5.0	3.0	3.8	0.1258	0.1813
	2 hours after	4	6.0	2.5	4.2	0.3746	0.4247
	*1 hour before	4	6.0	2.0	4.5	0.000	0.0547
	1 hour after	4	12.0	4.0	8.7	0.2280	0.2809
	2 hours after	4	12.0	8.0	10.0	0.4220	0.4730

* 5 gm. NH_4Cl were added to the regular diet.

† Gastrin injected.

THE AMMONIA OF THE GASTRIC JUICE OF DOGS

The gastric juice used in these determinations was collected from the accessory stomach pouch prepared according to the method of Pavlov. The collections were made in the morning for an hour period before feeding and for hour periods after feeding, the character and amount of food being a fixed constant during the entire observation period. The method of collecting the juice was the same as that used by Hardt (15) in his work on experimental ulcers in dogs. The ammonia was separated from the fresh juice by the Folin (8) aeration method and the amount determined by the Nessler colorimetric method as modified by Gulick (14). The results on normal dogs are summarized in table 1.

This table shows that the ammonia content of the pure gastric juice of normal dogs varies greatly in different dogs and in the same dog at different times. These findings are in accord with those recently reported by Carlson (3). In dogs that were fed a protein diet there was no uniform increase in ammonia content during the digestion period even in secretions collected from four to seven hours after feeding. When small amounts (5 gram) of NH_4Cl were added to the protein diet there was an increase in the ammonia content in most cases. This increase was usually most marked during the second and third hour after feeding.

THE AMMONIA IN THE GASTRIC JUICE OF DOGS WITH VARIOUS PATHOLOGICAL CONDITIONS

TABLE 2

The ammonia in the gastric juice of pathological dogs

DOG NO.	MATERIAL	NO. OF OBSERVATIONS	NH_3 IN MGM. PER 100 CC.			ACIDITY		
			Maximum	Minimum	Average	Free	Total	
10	Hunger juice	2	4.0	0.5	2.2	0.2630	0.3336	Distemper (sniffles)
11	Hunger juice	4	0.5	0.2	0.3	0.3190	0.3992	Pancreatectomy
12	Hunger juice	1	16.0	16.0	16.0			Pancreatectomy plus acute gastritis
13	Hunger juice	4	3.0	1.5	2.1	0.1595	0.2325	Splanchnics cut
14	Hunger juice	5	48.0	3.0	27.0	0.2771	0.4048	Ulcer in pouch
15	Hunger juice	7	32.0	12.0	20.0	0.1230	0.2278	Ulcer in pouch
	Second hour	9	36.0	12.0	19.0	0.2535	0.3319	
	Third hour	3	40.0	18.0	26.0	0.3326	0.4310	

At autopsy Dog 12 showed a very hyperemic gastric mucosa but no definite ulcer. Dog 14 was used by Mr. L. L. Hardt (15) in work on experimental gastric ulcer. On the first day of observation the total acidity was 0.3144 per cent and the NH_3 content 3 mgm. per 100 cc. of juice. Three days later the dog showed signs of distemper and refused to eat. The acidity at that time was 0.4174 per cent and the NH_3 content 24 mgm. Juice collected one hour before death showed an acidity of 0.3921 per cent and an NH_3 content of 48 mgm. At autopsy a definite ulcer was found in the Pavlov pouch.

Dog 13 also was used by Mr. L. L. Hardt in his gastric ulcer work. The first determinations thirty-two days before death showed a total acidity of 0.3644 per cent and an NH_3 content of 12 mgm. per 100 cc. of juice. Several days before being killed this dog refused to eat and from that time on there was a progressively increasing NH_3 content while the acidity remained at the same level. The day of death the total acidity was 0.4310 per cent and the NH_3 content was 40 mgm. At autopsy the Pavlov pouch showed a distinct almost healed ulcer but no other lesions could be found.

THE AMMONIA IN THE GASTRIC JUICE OF NORMAL MEN

The gastric juice used in these determinations was collected, except from Mr. V., the gastric fistula case, by means of the Rehfus (28) stomach tube. Three collections were usually made: first, the empty stomach; second, one-half hour of continuous secretion; third, one hour after taking an Ewald meal consisting of two pieces of dry toast and 200 cc. of distilled water. The determinations were made on samples from these collections by the method described above, except that in the Ewald meal collection a filtration was necessary before aeration could be satisfactorily accomplished. The findings for normal gastric juice are given in table 3.

All of these men were to the best of their knowledge in perfect health and all except V. and C. used the Rehfus tube on themselves almost daily in another line of experiments. A comparison of these results with the results found in normal dogs shows similar variations, but the findings are somewhat higher in man. In each case the Ewald meal juice shows the highest acidity and the lowest ammonia content.

TABLE 3

The ammonia in the gastric juice of normal men

INDIVIDUAL	MATERIAL	NO. OF OBS.	FREE ACIDITY	TOTAL ACIDITY	NH_3
			per cent	*per cent*	
Mr. V	Hunger juice	3	0.1643	0.2285	0.5
	Continuous juice	4	0.2914	0.3826	3.5
	Appetite juice	4	0.3642	0.4106	2.5
Mr. D	Hunger juice	5	0.0228	0.0729	12.0
Mr. H	Hunger juice	6	0.1897	0.2380	9.0
	Continuous	6	0.1979	0.2371	9.0
	Ewald	6	0.2152	0.2544	4.0
Mr. M	Ewald	3	0.1826	0.2006	3.0
Dr. E	Ewald	3	0.0738	0.1322	0.5
Mr. L. D	Ewald	2			3.0
Mr. L. H	Hunger juice	2	0.0784	0.1240	3.0
	Ewald	2	0.2169	0.2716	1.5
Dr. C	Hunger juice	3	0.0273	0.0547	16.0
	Continuous	4	0.0000	0.0474	14.0
	Ewald	3	0.0064	0.0711	12.0

THE AMMONIA IN THE GASTRIC JUICE OF MAN IN GASTRO-INTESTINAL DISEASES

The gastric juice employed in this work was collected with the ordinary stomach tube from individuals with gastric disturbances. The collections were made in the morning: first, aspiration of the empty stomach; second, aspiration one hour after taking an Ewald test meal consisting of two pieces of bread and 200 cc. of water. The ammonia in these samples was determined by the method described above. Observations were made in some of the individuals at the time of entrance into the hospital and in others after they had been on the ulcer treatment as outlined by Sippy (33) for a short time. Table 4 shows the average result of the findings with the probable or definite diagnosis in each individual case.

In all cases except one (22) the acidity of the Ewald meal juice exceeded the acidity of the plain aspiration juice but the ammonia content had no constant relation to the acidity and was not altered by a long stay in the hospital under treatment. All patients who had a relatively high NH_3 content for a period of several days were found to have either an ulcer or a carcinoma of the stomach.

TABLE 4

The ammonia in gastric juice of man in gastro-intestinal disorders

HISTORY	NO. OF OBSERVATIONS	MATERIAL	ACIDITY		NH_3
			Free	Total	
1. Mr. K. Age 26. Entered on April 29. Observed from May 9–26. Diagnosis, peptic ulcer	7	Aspiration	0.0552	0.1221	1.1
	4	Ewald	0.1744	0.2655	1.1
2. Mr. L. Age 33. Entered April 12. Observed from May 9–13. Diagnosis, peptic ulcer	3	Aspiration	0.0350	0.0847	1.0
	2	Ewald	0.1906	0.2703	1.2
3. Dr. B. Age 25. Entered April 20. Observed from May 9–11. Diagnosis, peptic ulcer, tooth abscess	2	Aspiration	0.0301	0.0637	1.2
	1	Ewald	0.1960	0.2507	1.5
4. Mr. N. Age 31. Entered March 30. Observed from May 9–26. Diagnosis, peptic ulcer, alveolar abscess. Roentgen diagnosis, duodenal ulcer	3	Aspiration	0.0926	0.1361	2.2
	3	Ewald	0.1966	0.2766	2.5
5. Mr. L. Age 53. Entered April 14. Observed from May 17–29. Diagnosis, peptic ulcer	6	Aspiration	0.1319	0.2227	0.5
	4	Ewald	0.2079	0.2779	0.5
6. Mr. A. Age 38. Entered May 10. Observed from May 13–31. Diagnosis, peptic ulcer	7	Aspiration	0.0636	0.1798	1.7
	6	Ewald	0.1527	0.2267	0.7
7. Miss B. Age 21. Entered May 7. Observed from May 29–June 4. Diagnosis, peptic ulcer	1	Aspiration	0.0136	0.0637	0.5
	3	Ewald	0.1857	0.2534	1.7
8. Miss G. Age 20. Entered May 23. Observed from June 2–4. Diagnosis, peptic ulcer	2	Aspiration	0.0781	0.1209	0.5
	1	Ewald	0.1230	0.2506	0.5
9. Mr. N. Age 47. Entered June 1. Observed June 7–9. Diagnosis, peptic ulcer	4	Aspiration	0.1558	0.1955	0.6
	1	Ewald	0.1851	0.2598	1.0
10. Mr. A. Age 31. Entered June 1. Observed June 7. Diagnosis, peptic ulcer	2	Aspiration	0.1882	0.2585	1.5
11. Mr. C. Age 34. Entered May 13. Observed June 8. Diagnosis, peptic and duodenal ulcers	1	Aspiration	0.0683	0.1321	0.2

TABLE 4—Continued

HISTORY	NO. OF OBSERVATIONS	MATERIAL	ACIDITY		NH_3
			Free	Total	
12. Mr. K. Age 23. Entered May 27. Observed June 7. Diagnosis, peptic ulcer	1	Aspiration	0.1532	0.2416	4.0
13. Mr. R. Age 46. Entered May 24. Observed May 26. Diagnosis, peptic ulcer	1	Aspiration	0.0820	0.1321	6.0
14. Mr. S. Age 42. Entered May 13. Observed May 26–30. Diagnosis, peptic ulcer with obstruction. Gastro-enterostomy, ulcer found	2	Aspiration	0.00	0.1180	16.0
15. Mr. G. Age 27. Entered June 15. Observed June 16. Diagnosis, peptic ulcer	1	Ewald	0.1750	0.2744	12.0
16. Mr. C. Age 32. Entered June 15. Observed June 16. Diagnosis, peptic ulcer	1	Ewald	0.1641	0.2452	1.0
17. Mr. H. Age 35. Entered June 15. Observed June 16. Diagnosis, peptic ulcer	1	Ewald	0.1732	0.2981	2.0
18. Mr. M. Age 21. Entered June 16. Observed June 17. Diagnosis, peptic ulcer	1	Ewald	0.0985	0.2353	3.0
19. Mr. P. Age 46. Entered June 15. Observed June 17. Diagnosis, tabes	1	Ewald	0.1167	0.3054	12.0
20. Mr. E. Age 20. Entered April 8. Observed May 17. Diagnosis, tuberculosis of bronchial glands	1	Ewald	0.2170	0.2735	1.5
21. Mr. F. Age 66. Entered June 7. Observed June 8. Diagnosis, carcinoma of stomach	1	Ewald	0.00	0.0695	1.0
22. Mrs. B. Age 42. Entered April 28. Observed May 9–20. Diagnosis, carcinoma of stomach, confirmed by operation	4 4	Aspiration Ewald	0.0408 0.0121	0.0788 0.0321	4.9 4.6
23. Mrs. M. Age 59. Entered. Observed July 7. Diagnosis, carcinoma of stomach	4	Aspiration	0.00	0.0604	13.0

THE INFLUENCE OF VARIATIONS IN THE DIET ON THE AMMONIA OF GASTRIC JUICE

In order to determine some of the factors that influence the NH_3 content of the gastric juice a series of experiments was conducted on one of the normal individuals (Mr. H.), who showed a constant high NH_3 content. During these experiments the gastric juice was collected twice each morning before breakfast. The stomach was first emptied with the Rehfus tube, then washed thoroughly with distilled water and emptied again. A second collection was made after one-half an hour of continuous secretion. The free and total acidity and the NH_3 content of both collections were then determined. In order to determine the relation of the NH_3 content of the gastric juice to the NH_3 content of the urine, determinations of the NH_3 content and of the total N of the urine were made on the total urine for each day. The NH_3 of the urine was determined by the method described above and the total N was determined by the Kjeldahl-digestion-distillation-titration method.

In the first experiment the diet was so arranged that there was a period of high protein and a period of low protein ingestion. In the second experiment the diet was kept uniform but during certain periods an excess of base- or of acid-forming radicals was added in water solutions. The base-forming radicals were increased by using the treatment for gastric ulcer as outlined by Sippy (33). The acid-forming radical was increased by adding phosphoric acid, which is a normal constituent of the protein molecule. The complete results of the two experiments are recorded in tables 5 and 6.

The low protein diet consisted of potatoes, rice, fruit, cream and a small amount of bread. There were no noticeable symptoms during the period of ingestion of this diet.

The high protein diet consisted almost wholly of meats—fish, steak, liver, sweetbreads, etc. with a small allowance of bread. No fats were ingested. The symptoms during this period were very noticeable; first day, nothing noticeable; second day, easily fatigued on exertion; third day, easily fatigued on slight exertion, joints and muscles aching, could work only a short time without rest. On this day the urine contained many uric acid crystals; fourth day, symptoms of third day more pronounced. Very sleepy and depressed. Urine contained a heavy precipitate of uric acid.

These symptoms cleared up quickly when the mixed diet was resumed. The absolute increase or decrease in the urinary NH_3 follow-

TABLE 5

The influence of variation in protein content of diet on the ammonia of the gastric juice

DATE	GASTRIC JUICE			URINE			
	Acidity		NH_3	Volume	Total N	Total NH_3	NH_3
	Free	Total					
Ordinary diet							
						mgm.	*per cent*
July 10........	0.0638	0.1094	16	800	4.1664	320.0	7.69
July 11........	0.0665	0.1020	16	650	3.735	292.5	7.83
Low protein diet							
July 12........	0.1066	0.1713	8	680	3.843	258.4	6.71
July 13........	0.1148	0.1494	7	610	4.833	129.6	2.66
July 14........	0.1531	0.2078	5	1620	5.493	162.0	3.71
July 15........	0.2598	0.2871	3.5	760	6.719	243.0	3.62
Ordinary diet							
July 16........	0.0337	0.0820	12	960	10.666	384	3.60
July 17........	0.1395	0.2033	12	680	9.203	380.8	4.13
July 18........	0.1021	0.1349	14	895	11.335	319.2	2.81
July 19........	0.0729	0.1258	16	1080	11.83	361.8	3.06
July 22........	0.1303	0.1740	10	990	9.41	356.4	3.78
High protein diet							
July 23........	0.1167	0.1659	10	1155	19.36	369.6	1.905
July 24........	0.1395	0.1851	29	1420	24.54	426.0	1.734
July 25........	0.1003	0.1413	24	1530	27.86	550.8	1.977
July 26........	0.1321	0.1658	26	1510	24.04	815.0	3.388
Ordinary diet							
July 27........	0.0656	0.1002	19	1110	14.02	799.2	5.970
July 28........	0.0747	0.1148	12	1500	9.91	600.0	6.053
July 29........	0.0638	0.1003	10	1365	9.86	435.0	4.411

ing increase or decrease in the protein content of the diet is more marked than is usually observed. We are unable to give a satisfactory explanation for this variation.

TABLE 6

The influence of variations in acid and basic-radical content of diet on the ammonia of gastric juice

DIET	DATE	GASTRIC JUICE			URINE			
		Acidity		NH_3	Volume	Total N	Total NH_3	NH_3
		Free	Total					
								per cent
Ordinary diet.....	August 15	0.0610	0.1003	16	800	9.576	394.32	4.11
	August 16	0.0182	0.0547	14	1000	11.599	448.8	3.87
	August 17	0.0783	0.1211	10	1215	9.460	427.6	4.52
Ordinary diet plus alkali. Per day: 50 gr. calcined Mg.; 150–300 gr. $NaHCO_3$; 75 gr. bismuth subcarbonate; Bi_2O_3-CO_2H_2O.........	August 18	0.1368	0.1687	10	1570	8.715	415.6	4.76
	August 19	0.1185	0.1659	10	1715	9.24	429.0	4.64
	August 20	0.1832	0.2260	9	1705	8.21	351.0	4.27
	August 21	0.0939	0.1349	10	1470	8.448	237.0	2.80
	August 22	0.1686	0.1956	12	2035	8.632	105.4	1.22
	August 23	0.0519	0.0820	14	1365	8.738	40.2	0.46
Ordinary diet.....	August 24	0.0747	0.1048	12	1220	8.569	106.75	1.24
	August 25	0.0620	0.0866	12	1260	10.900	146.28	1.34
	August 26	0.1777	0.2296	10	1530	15.492	432.32	2.98
	August 27	0.0547	0.0866	12	2135	10.259	339.7	3.31
	August 28	0.0865	0.1138	12	1290	9.476	360.0	3.97
	August 29	0.0912	0.1185	12	1050	10.574	285.6	2.70
	August 30	0.1003	0.1413	13	1580	11.336	364.2	3.21
	August 31	0.2006	0.2325	12	1280	9.873	375.6	3.79
Ordinary diet plus acid. Per day: 15 to 20 cc. of a 4 per cent syrup of phosphoric acid...........	September 1	0.1230	0.1458	9	1315	9.336	333.6	3.48
	September 2	0.1021	0.1322	10	1635	8.402	310.8	3.69
	September 3	0.1641	0.1987	11	1070	7.179	414.3	5.77
Ordinary diet.......	September 4	0.0838	0.1157	12	1295	9.590	368.3	3.83

During the period of ingestion of a large excess of alkali radicals there were no noticeable symptoms but the total acidity of the urine was greatly diminished. During the period of ingestion of excess of acid radicals there was a feeling of discomfort for two hours after taking each dose. No other symptoms were noted. On the last day of this acid-radical ingestion the total acidity of the urine was markedly increased.

In all of the work on normal or pathological man the juice collected frequently contained varying amounts of bile but the NH_3 content of these samples did not seem to be influenced.

THE AMMONIA CONTENT OF THE GASTRIC MUCOSA

Salaskin (31), Horodynski, Salaskin and Zaleski (17) by using the Nencki-Zaleski method of NH_3 determination found a high percentage of NH_3 in the contents and mucosa of the stomach and intestines of animals. As the presence of this relatively high percentage of NH_3 in both the contents and mucosa of these organs suggested a connection with their secretory processes some NH_3 determinations were made by the newer methods on these tissues in dogs. Four dogs that had been fasted from fifteen to forty-five hours, and one that had been fed only two hours previously, were killed by bleeding. The stomach and duodenum were immediately removed, opened and washed thoroughly with distilled water. Eight to ten grams of the mucosa was stripped from the cardiac, fundic and pyloric regions of the stomach and from the duodenum, and these portions of mucosa were pulverized in a mortar with purified sea sand. These pulverized masses were extracted several times with warm distilled water which was slightly

TABLE 7.

NH_3 content of gastric and intestinal mucosa in dogs. Per 100 grams fresh mucosa

DOG	I	II	III	IV	V	AVERAGE	AMOUNT FOUND BY ZALESKI-NENCKI AND PAVLOV
Hours without food	2	15	18	19	45		
NH_3 mgm. in 100 grams cardiac mucosa	0.60	1.41	0.50	0.46	0.22	0.64	
NH_3 mgm. in 100 grams pyloric mucosa	1.78	2.65	1.67	0.62	1.04	1.55	37.1–52.8
NH_3 mgm. in 100 grams fundus mucosa	1.2	4.63	5.25	1.40	5.22	3.54	
NH_3 mgm. in 100 grams duodenal mucosa	1.1			0.94	0.90	0.97	23.0–41.7

Nencki, Pavlov and Zaleski made their determinations on the mucosa from animals that had been fed two to nine hours before being killed. Horodynski, Salaskin and Zaleski obtained approximately the same results.

acidulated with acetic acid, the washings for each portion combined, boiled for five to ten minutes, and filtered. The acid filtrate was evaporated on the steam bath to about 15–20 cc. and the NH_3 content determined by the methods described above.

DISCUSSION

The data presented above shows that ammonia is a constant constituent of the gastric juice of man and dog and that the amount found varies between rather wide limits, especially in man. Many investigators, Sticker (35), Sommerfeld (34), Reissner (27) and others, have contended that this ammonia has its origin in the saliva and Boldyreff (1) has shown that the duodenal content is often regurgitated into the stomach. Strauss (36), and more recently Carlson (3) eliminated one or both of these possible sources of contamination by using the juice collected from men with a gastric fistula or from dogs with a Pavlov stomach pouch. Their work showed that ammonia was constantly present in pure gastric juice. Rosenheim (31) concluded that the ammonia came from the secretions of the stomach itself as it was present in approximately the same amount regardless of the time of test or state of digestion. Rosemann (29) thought that the ammonia must be a product of cell activity as it was in higher concentration in the gastric juice than in the blood. Zunz (40) found by analyzing stomach contents and test meals that there was no relation between the amount of ammonia and the state of digestion or the concentration of the hydrochloric acid. The present work is in accord with and corroborates these findings of Strauss, Carlson, Rosenheim and Zunz and shows further, that the ammonia content of the gastric juice of man may be increased or diminished by varying the protein content of the diet. During this variation in diet there is a corresponding variation in urinary ammonia. Loeb (23), Schittenhelm (32), Grammeltoft (13) and others, have shown that urinary ammonia decreases in amount during the height of gastric secretion. Although these facts seem to point to a possible relation between the occurrence of ammonia in the urine and in the gastric juice, the controlling mechanisms are influenced differently by at least one factor, i.e., the ingestion of an excess of acid- or base-forming radicals. In this case the ingestion of an excess of base-forming radicals causes a marked decrease in urinary ammonia and the ingestion of an excess of acid-forming radicals has the opposite effect, but neither of these has any effect on the amount of gastric juice ammonia. It was also noted that in man during a period of fasting lasting from twenty-four to forty hours the ammonia content of the gastric juice decreased progressively, but that during the height of absorption of a mixed or protein diet in dogs there was no corresponding increase except in some cases where NH_4Cl was added to the diet. However, this last observation is not altogether trustwortny as Underhill and Goldschmidt (37) have found that "the ingestion of ammonium chloride may be regarded as a distinct detriment to nutritional rhythm" and they believe that ammonium chloride acts as a stimulant to protein katabolism.

During recent years much work has been done on the fluids originating in the gastro-intestinal tract and some of these observations are of especial interest in the present work. It has been shown by different workers that the mucosa of the alimentary tract may act as an excretory organ for such substances as Fe, Ca, PO_4, Hg, various pigments, mono-saccharides, etc.[1] Voit (38) and Hermann (16) were among the first to show the importance of the nitrogen content of the fluids collected from the alimentary tract. Corlette (5) concluded that the excretion of nitrogen from the bowel wall did not vary according to general metabolism in the same way as occurred in the kidneys. This observation is in accord with our results. Mosenthal (25) while studying the secretion of a Thiry fistula of the lower ileum in dogs estimated that the total nitrogen secreted by the small intestine varied between 225 to 607 per cent of that found in the feces, the greater part of this apparently being reabsorbed. Of this nitrogen, about 1.5 to 3.25 per cent was in the form of ammonia. He states further that the intestine has at least three functions, namely; excretory, digestive including absorption, and a third, that of forming a link in the "circulation" of various products, such as nitrogen or the bile pigments. We made some ammonia determinations on juice collected from a Thiry fistula of different parts of the small intestine of dogs and found that the ammonia ranged between 1.5 to 12 mgm. per 100 cc. of juice.

Another point of interest in this connection is the observation made by Cohnheim (4) that the intestinal mucosa of fishes has the power to deaminize proteins. However, he was unable to extend this observation to mammals although Jacobi (18) and Lang (21) had shown that tissue extracts of the intestine were able to deaminize amino-acids.

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[1] The occurrence of ulcers in the duodenum following extensive superficial burns and in the colon in the late stages of uremia gives further evidence of this excretion.

In Folin's (12) original theory of protein metabolism the assumption is made that the intestinal mucosa has the power of deaminization. Abderhalden favors the view that the protein molecule is broken down completely in the intestinal mucosa. Later, Folin (9) concluded that the intestinal mucosa had no special deaminizing power for he was able to show that the ammonia content of the portal blood was directly dependent on the putrefactive processes in the large intestine. Folin (10) also showed definitely that there is an absorption of protein digestion products by the stomach mucosa. It should be noted that the workers on the mechanism of intestinal absorption and metabolism have taken into account only those products found in the efferent blood or lymph vessels and have neglected altogether those products which pass back into the lumen of the intestine.

There are certain factors which speak against considering the gastric juice ammonia as due to either a normal secretory or excretory process. Although ammonia is constantly found in all samples of normal gastric juice, it is evident that the mechanism governing the production of this constituent is not the same as that governing the acid or pepsin production, for the concentration of the ammonia has no relation to the concentration of the acid or pepsin, and there seems to be no relation between the ammonia and the amount of mucus. The similar variations in concentration of urinary and gastric juice ammonia which may be brought by varying the N-content of the diet seem to point to an excretory process, but the mechanisms controlling these processes are different, for the urinary ammonia may be made to vary markedly by other means without affecting the concentration of the gastric juice ammonia.

Zunz (40), working on stomach contents and test meals, showed that in some pathological cases the ammonia nitrogen was appreciably increased but he was unable to account for this increase. A few years before Emerson (6) demonstrated an enzyme in malignant growths which was capable of splitting protein beyond the albumose stage. Jacque and Woodyatt (19) also showed that carcinomatous stomach contents had, in 88 per cent of cases examined, a peptolytic power two to ten times the maximum seen in benign conditions and suggest that a part of this is due to bacteria, as the degree of proteolysis was lowered somewhat by filtration through a Berkefeld filter. Folin, as was noted above, showed a definite relation between ammonia content of the portal blood and bacterial decomposition in the large intestines. Recently, Burgett has isolated a fairly constant flora from the pure gastric juice of Mr. V., the gastric fistula case. Kendall and Farmer (20) have reported that many bacteria have a deaminizing power which is exercised to a marked degree if carbohydrate is lacking or small in amount.

It seems then that enzymatic or bacterial action on the stomach mucosa or on the gastric juice may be responsible for some of the ammonia found in the gastric juice in some cases, but the constancy of the ammonia content of the fresh juice of normal man and dog speaks against this as the sole or principal origin.

The diagnostic significance of a high content of ammonia in the gastric juice in cases of ulcer or gastric cancer is made questionable by the finding of an equally high content in the juice of some apparently normal individuals.

It was observed that some of these cases (hospital patients and sick dogs) with high ammonia values showed a marked cachexia. Observations are being made to determine whether any relation exists between cachexia, acidosis and the ammonia content of the gastric juice. Folin's (11) new method of NH_3-determinations by direct Nesslerization is being used in this work.

CONCLUSIONS

1. Ammonia is normally found in small amounts in the gastric juice of man and dog.
2. The amount of ammonia in the pure gastric juice of man varies markedly in different individuals. It is increased by high protein diet, and by the addition of ammonium salts to the food.
3. The fundic mucosa contains the most ammonia, the pyloric mucosa is next in order, while mucosa of the cardiac end of the stomach contains the least ammonia. The duodenal mucosa contains less ammonia than the fundic mucosa.
4. In certain pathological conditions (especially gastric ulcer and cancer) the amount of ammonia in the gastric juice is definitely increased, but this increase may not be greater than that found persistently in certain normal individuals.
5. Our results indicate that the origin of the gastric juice ammonia is a complex one. Some of the ammonia is evidently excreted from the blood, and this factor depends on the concentration of the ammonia in the blood. Part of the ammonia represents deaminization processes in the gastric mucosa, and these in turn depend on the quan-

tity of protein in the food. The possible rôle of the gastric flora has not been eliminated.

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VI. DIFFERENCES IN LATENT PERIOD AND FORM OF THE CONTRACTION CURVE IN MUSCLE STRIPS FROM DIFFERENT PARTS OF THE FROG'S STOMACH

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It has been shown in a recent paper that strips of muscle cut from different parts of the wall of the mammalian stomach vary markedly in their tendencies to rhythmic contraction, and in the forms of the curves obtained (1). Similar differences were found in the behavior of circular strips from the cardiac and pyloric regions of the frog's stomach. Marked variations were found also in the irritability and latent period when different parts of the stomach were stimulated with the same strength of current (2).

The question now arises, what is the origin of these differences? Are there peculiarities of structure and metabolism behind them, and if so, are they in the muscle, in the nerves, or in both? The fact that the strips would often beat better on the second or third day after excision than on the first, and that they would show some rhythmicity even after five days, made it hard for me to accept Magnus' view that the rhythm is dependent upon Auerbach's plexus (3). Although the myenteric nerve cells are peculiarly resistant (4), one can hardly imagine their functional capacity increasing forty-eight to seventy-two hours after death. It seems probable enough that the nerve net regulates and profoundly influences the muscle, but recent work on the cultivation of tissues has shown conclusively that it is not essential to the institution of rhythmic contraction. Muscle cells will beat in the test tube when there can be no question as to the absence of nervous tissue (5). Recently Gunn and Underhill (6) have repeated Magnus' work, and have obtained rhythmic contractions in strips from which it would seem certain that all remnants of the plexus had been removed. Their pharmacological studies, using nerve-ending and muscle stimulants at varying intervals after the removal of the strips showed that the muscle lost its rhythmicity when it seemed to die, that is, when it no longer reacted even to barium chloride. This was usually after four or five days. It would seem best, then, to look for peculiarities in the muscle itself, peculiarities that would explain the different behavior of the strips from the different regions.

The rate of rhythmic contraction seems to be suited to the metabolism of the muscle. As Woodworth says (7), "Both activity and rest are good for a muscle. If the pause preceding a contraction is too short, the contraction suffers from lack of recuperation. If it is too long, the contraction suffers from what we may perhaps call a sort of drowsiness." If we speed up the metabolism of the muscle by warming it, the rate of rhythmic contraction is increased; and with artificial stimuli, the latent period is shortened, and the form of the curve greatly changed. These changes are well shown by Stewart (8). They probably account for De Zilwás (9) finding that, with the muscle at 40°C., stimuli had to follow each other within from five to ten seconds if summation was to be obtained. At 25°C. the interval could be twenty seconds or more. Similarly, Engelmann (10), while studying the ureter of the rabbit, found that a series of subliminal stimuli might bring about a contraction if the interval between them was not too great for summation. When the irritability was high and the waves were traveling rapidly down the ureter, the interval had to be shorter. It had to be shorter also when using the ureter of the rat, as there the rate of the waves is normally faster than in the rabbit. Woodworth (11) found that the smallest interval at which two stimuli can come, so that the effect of each shall show separately on the curve, varies widely in stomach strips from different frogs. It seems to me, from all this, that when we find, in the same animal, a strip of duodenum contracting twenty times a minute and a strip of ileum contracting ten times, we should expect to find a more active metabolism in the one than in the other. The muscle in the duodenum may be different from that in the ileum, much as the pectoral muscles of a bird are different from the thigh muscles.

Ranvier (12), years ago, commented upon the anatomical differences in smooth muscle from the aorta, from the arterioles, intestine, bladder, uterus, etc. In the arteries and in the intestine the fibers are generally quite parallel; in the bladder of the frog there is a marked reticulum, formed by the interlacing of tiny bundles of muscle. McGill (13) has shown that there persists in various parts of the digestive tract an embryonic type of smooth muscle which can be recognized by its branching fibers and syncytium formation. Grützner (14) warns us not to take strips from the region of the pylorus in the frog, because he says the fibers are not parallel there but interlace diagonally. He says it is well known that different examples of smooth muscles (from different animals and different organs) have different irritabilities, different latent periods, and forms of the contraction curve. See table on page 56 of his article.

Pompilian, also, (15) found big differences in latent period and duration of contraction between the smooth muscles of the eye-tentacles and those of the foot of the snail. To the naked eye, the tentacle muscle is paler than that of the foot. He could not make out histological differences, but he felt they must be there. Lapicque (16) showed that the duration of stimulus necessary to bring about a contraction varies markedly in different examples of muscle from lower animals. I can find no reference to differences in muscle in different parts of the digestive tube. The nearest approach to it is the opinion expressed by some writers that the irregular contractions met with were due to an interference of waves originating in fibers of different rhythmicity (17). Thus, in the esophagus of the toad, Bottazzi (18) found that the longitudinal fibers contract more frequently than do those of the circular muscle.

It must not be forgotten that there may be physiological differences also in the nerves associated with these muscles. Local anatomical differences in the nerve net have been commented upon in previous papers (19). Carlson's work (20) suggests that more irritable and rapidly conducting nerves will probably be found in connection with the quicker muscles.

It was this literature which encouraged me to look for differences underlying the big differences in rhythmicity which I have found in various parts of the digestive tract. I wished to see also if the muscle strips from different regions would retain those peculiarities of irritability, latent period and form of contraction observed in the intact stomach, and described in the last paper. Work was done first on the stomach of the frog on account of its simplicity. Most of the writers on smooth muscle have used circular strips from the frog's stomach, but I can find no reference to differences in the behavior of strips from different parts of the organ. The middle strip has generally been used. Schultz (21) says he used pieces from a little below the middle, and his tracings show that they must have come from the upper antrum. It should be explained here that the pyloric end of the frog's stomach is different from the cardiac end. It is firmer to the touch; the wall is thicker, and its outer surface is smooth and glistening.

TECHNIC

Narrow strips were obtained from the cardia, the middle region, and the pyloric antrum. Several difficulties had to be overcome before a satisfactory technic could be worked out. Since differences have been observed in the behavior of strips with and without the mucosa (22), it seemed best to preserve it carefully at all times so that the conditions would be more comparable. No difficulty was encountered in cutting strips from the cardiac region, as the mucous membrane is there closely adherent. Near the pylorus, however, the greatest care had to be taken or the mucosa would slip off and be lost. This laxity of the submucosa in the antrum pylori was marked in the higher animals also. Most of the rings were cut with a razor, and then carefully opened out. They were from 3 to 5 mm. wide. Those from the cardiac end were longest, but this disparity in length could apparently be disregarded, as the cardiac strips reacted the same, before and after being trimmed to the length of the pyloric ones.

These strips were suspended in a moist chamber similar to that described by Porter (23). It was made from a six ounce specimen bottle lined with blotting paper. This bottle was kept in a water bath at 19°C. The light heart lever was weighted with 1 gram; just enough to balance the preparation and to keep it taut. The faradic current was used. It was obtained from a Harvard Apparatus Company's inductorium (24) with one dry cell, and was led to the two little serrefines grasping the ends of the strips. Each strip was stimulated first with the secondary coil at 6 cm. and later with it at 4 cm. No reactions could be obtained with the coil at 8 cm. The duration of the stimulus was about one second; longer with the weaker current and more sluggish strips, shorter with the stronger current and more irritable strips. Such slight differences in stimulation did not seem to have any effect on the latent period or upon the form of the contraction curve. Some work was done with strong make and break induction shocks. Only the break seemed to have any effect, and this was much less marked than that of the tetanizing current. The latent period was longer with the single shock, but otherwise the shapes of the contraction curves were the same. Five minutes were generally allowed between each stimulus for the recovery of the muscle. It was found later that this interval might have to be longer when studying the strips from the cardia, and that it might be much shorter for the antral strips. California frogs were used, rana draytonii and rana temporaria. As was to be expected, there were marked individual differences, due probably to infection with parasites, condition of the reproductive organs, etc. The poorest strips came from some animals that were left over night in a steam-heated room. Most of the work was done between October and January. Better results were obtained in six frogs studied about May 1. Their stomachs generally contained food, and they were more active than were the winter frogs.

LATENT PERIOD

Figure 1 represents the latent periods obtained in studying strips from the two ends of the stomach in nineteen frogs. The lines connect successive readings on the same strip. The first and third columns represent figures from the cardia; the second and fourth represent those from the antrum. The first two columns of figures were obtained with the secondary coil at 6 cm.; the next two were obtained with the coil at 4 cm. The strips were always stimulated with the weaker current first, as the stronger stimuli often seemed to injure the muscle.

It will be seen at a glance that the latent period was longer and more variable in the cardia than in the antrum, and this was true for both strengths of current. The widest variations were seen in the cardiac strips when stimulated with the coil at 6 cm. A number of factors may have been active in bringing out these differences. In the first place, the cardia seemed to suffer much more from the trauma of excision than did the antrum. Recovery from this trauma was seen in the progressive shortening of the cardiac latent periods for some time after attachment. Typical examples of this are shown in the following table. The figures represent seconds.

TABLE 1

∞ ; 4.2; 3.4; 2.7; 1.4; spontaneous contractions; 4.6
∞ ; 2.2; 2.3; 1.5
∞ ; 8.0; 4.0

The first experiment is particularly instructive, as the strip which at first failed to react at all, after 25 minutes, reacted in 1.4 seconds. The latent period of the corresponding strip from the antrum was, in successive tests, 4.8, 4.3, and 4.0 seconds. Here, again, improvement is to be noted but it is not so marked as in the cardiac strip. In a number of instances, the three or four successive tests on an antral strip gave the same figure for the latent period. This is shown by the number of horizontal or nearly horizontal lines in the second and fourth columns of figure 1. I have commented elsewhere on a similar difference in vulnerability between segments from the duodenum and from the ileum (25). When a number of such segments are put into

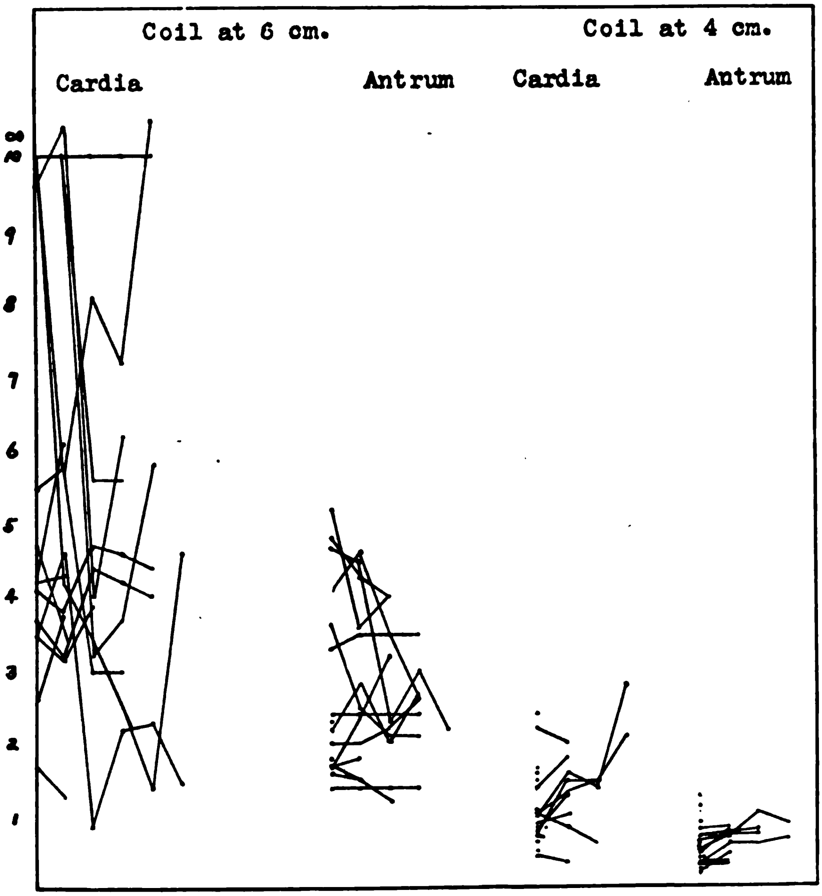

Fig. 1. Latent periods of cardiac and pyloric muscle strips from the stomachs of nineteen frogs. Ordinates represent seconds; the lines connect successive tests on the same strip.

warm oxygenated Ringer's solution, those from the ileum will generally begin beating first, and they will stand much more trauma than will the more sensitive duodenal segments.

The great rhythmicity of the cardiac strips interfered considerably with the work. In the first place, it made the records difficult to read on account of the changing base line, and at times it was hard to say whether or not a rise after stimulation was wholly or in part spontaneous. A certain refractoriness often appeared with the onset of rhythmic contractions. This did not correspond to that observed with heart muscle, as a contraction could easily be superimposed upon any part of a spontaneous wave, but there was a lowering of irritability and a lengthening of the latent period. Returning to the table, it will be seen in the first protocol that the appearance of rhythmic movements was followed by a lengthening of the latent period from 1.4 to 4.6 seconds. Sudden alterations in irritability often gave me the impression that rhythmic changes were going on in the muscle although there were no visible contractions. These suspicions have been strengthened by Stübel's (26) observation that rhythmic action-currents can be obtained from the stomachs of birds even after the contractions have been inhibited.

A number of methods were tried in the hope of stopping the rhythmic waves without interfering with the irritability of the strips. Schultz's (27) method of removing the mucous membrane and soaking in atropin solution was tried on a number of frogs, but it seemed to injure the cardiac strips more than it did the antral ones, so it could not be used. Sometimes the movements could be inhibited by a series of subliminal stimuli. The best results were secured by stimulating just after the muscle had relaxed from an artificial contraction and before the spontaneous waves could begin again. No such difficulties were encountered with the antral strips, which showed very little rhythmicity.

Not only were there these rhythmic movements to contend with, but sometimes there were slow variations in tone. Jordan (28) believes that a change in tone greatly alters the irritability of smooth muscle, and it may be that the low irritability of the cardiac strips at first was due partly to their increased tone. Grützner (p. 27) quotes the literature to show that smooth muscle when tonically contracted will not react to the strongest induced currents. He found this true for freshly cut stomach rings; but if he killed the frog, left it in a cool room for 24 hours, and then cut a ring, it was much more irritable and reacted well. I repeated this work, and found that the improvement was to be noted only in the strips from the antrum. This subject will be taken up again later. I do not believe that changes in tone affected the reactions very much. The strips from the antrum showed the most even base line, just as they did when beating in Ringer's solution.

When we remember how much shorter the latent period was at the cardia than in the antrum of the intact stomach, it is surprising to find the conditions reversed in the strips. Excluding the negative results, the averaging of 172 readings in 21 frogs gave the following figures:

TABLE 2

	Cardia	*Antrum*
6 cm.	3.90 seconds	2.70 seconds
4 cm.	1.26 seconds	0.66 seconds

In only two frogs were the lowest figures obtained with the cardiac strip less than the lowest for the antral strip from the same stomach. The protocols are as follows:

TABLE 3

Frog	*Coil Distance*	*Cardia*	*Antrum*
1.	6 cm.	4.2; 3.4; 2.7; 1.4; 4.6	4.8; 4.3; 4.0
	4 cm.	1.60	1.15
2.	6 cm.	3.5; 4.6; 0.9; 2.2; 2.3; 1.5	2.4; 2.4; 2.4; 2.4
	4 cm.	0.43; 0.38	0.55; 0.75

The second protocol is very definite, as the results with the two strengths of current are in accord. This evidence that the cardiac strips can at times react more quickly than the antral ones raises the question as to whether they might not always do so if it were not for the greater sensitiveness to trauma, the greater rhythmicity and refractoriness. It will be seen later that, in the mammalian stomach, the latent period of the strip from the cardiac region was usually shorter than that of the antral strip. If, in the frog, the latent period of the *muscle* in the antrum is really much shorter than that around the cardia, it would seem that the greater irritability of the cardia in the intact stomach must be attributed to the activities of the nervous plexus. In the intact stomach, the cardia responds to stimulation with the coil at 13 cm. and the antrum with it at 8 cm. Excised strips from both regions react only when the coil is at 6 cm., hence the loss of irritability is greater proportionately in the cardiac strips. Since

Barbera's (29) results on the excised stomach agree closely with my own for the organ in situ, it would appear that the disproportionate lengthening of the latent period must be due more to the cutting of the strips than to the stoppage of the circulation and the severance of the extrinsic nerves.

DIFFERENCES IN THE REACTION TO TRAUMA

Several writers have commented upon the injurious effects of strong induced currents on smooth muscle. Apparently the cardiac strips suffered from this more than did the antral ones, judging by the degree of lengthening of the latent period after successive stimuli (with the coil at 4 cm.). Some of the antral strips did not seem to be injured at all, while a few of the cardiac ones lost their irritability entirely after one or two shocks. The following protocols of experiments with the secondary coil at 4 cm. bring this out.

TABLE 4

Cardia	*Antrum*
0.80; 1.30; ∞	0.32; 0.35
0.77; 1.50; 1.50; 2.10	0.35; 0.60; 0.60; 0.70
1.00; 1.60; 1.40; 2.80	0.72; 0.70; 1.15; 0.90
1.10; ∞	0.80; 0.85
2.80; 4.40; 6.40	1.40; 1.20; 2.10
0.85	0.75; 0.60; 0.50

This difference was most marked in diseased or weakened animals in which the cardia showed very little vitality. The impression was gained while studying the mammalian stomachs also, that the more sensitive cardiac muscle suffers most from a general lowering of the bodily strength and resistance. Perhaps for the same reason, the cardiac strips did not stand being kept in the ice box for twenty-four hours so well as did those from the antrum. After the pieces had been tested, they were put in Ringer's solution, usually at 6°C., and the reactions were taken again next day. Some kept well, and contracted with as good amplitude as they showed at first; others, usually from the weaker animals, reacted poorly. The following figures show that the latent period in the antral strips may even be shorter after twenty-four hours. This was observed quite constantly, later, with antral strips from the dog. The figures in the following table represent the shortest periods obtained during the usual series of tests on each strip.

TABLE 5

FROG	COIL DISTANCE	CARDIA			ANTRUM		
		After excision	After 24 hours	Per cent	After excision	After 24 hours	Per cent
	cm.						
1	6	2.60	3.20	123	2.30	1.70	73
	4	0.85	1.75	205	0.65	0.61	93
2	6	3.50	∞	∞	1.70	3.00	176
	4	0.65	2.20	338	0.40	0.30	75
3	4	2.00	3.90	195	1.30	1.80	138
4	4	1.50	3.40	226	0.55	0.85	154

After the 24 hour interval, the amplitude was much better in the antral strips than in the cardiac ones.

STRIPS FROM THE MIDDLE OF THE STOMACH

In eight frogs, a strip was cut from the middle of the stomach. All but one of these resembled in their reactions the corresponding cardiac strips, although the latent periods were sometimes shorter. As the frog's stomach has no distinct upper limit, its mid-point could not always be located with accuracy, and it may be that some of the strips were taken, really, from the upper edge of the pyloric antrum. These strips from the middle stretched out a great deal and did not seem to have much tone.

SHAPE OF THE CONTRACTION CURVE

A very definite difference was found between the type of curve traced by the cardia and that of the antrum (see fig. 2). The following table shows latent period, apex and relaxation times expressed in seconds elapsed after stimulation.

The better amplitude usually met with in the antral strips made it easier to get good tracings from them than from the cardiac ones. I have remarked elsewhere on the wide amplitude of the *spontaneous* contractions in the antrum. The relaxation time, particularly in the cardiac strips, seemed often to be shortened or lengthened according as the tone happened to be falling or rising. When it was rising, the lever generally did not return to its old level at all. After twenty-four hours, the relaxation time was sometimes shorter, sometimes longer. The slower relaxation of the cardiac strips may be due to the greater sensi-

TABLE 6

COIL DISTANCE	CARDIA			ANTRUM		
	Latent period	Apex	Relaxation	Latent period	Apex	Relaxation
cm.						
6	1.30	32	480	0.95	20	160
	3.20	29	265	1.90	16	50
	3.50	67	217	0.55	21	89
4	0.50	22	290	0.48	16	150
	0.85	30	480	0.20	13	190
	1.75	45	400	0.65	18	164
				0.61	18	114
				0.85	21	90
				0.40	17	206
				0.35	12	70
Averages.......	1.85	37	355	0.69	17	130

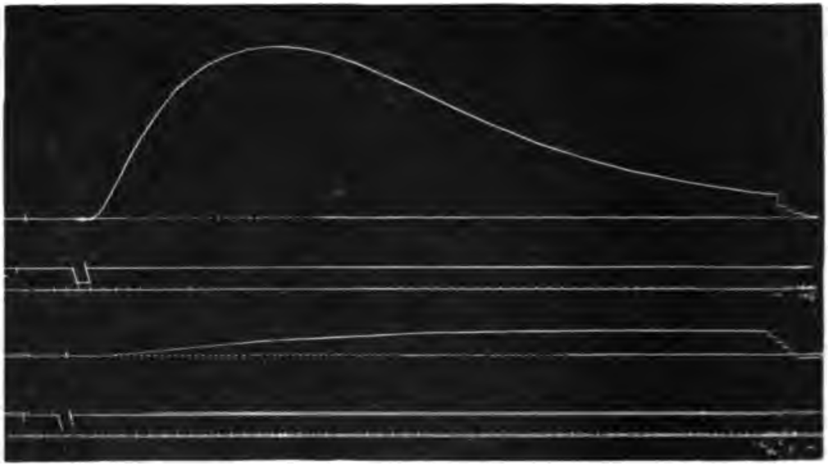

Fig. 2. Contraction curves from strips from the antrum (upper) and the cardia (lower). The same strength of faradic current was used. Time markings represent seconds. Magnification of lever 4.5:21.

	LATENT PERIOD	APEX	RELAXATION
Antrum................................	0.6	16	145
Cardia.................................	2.2	45	200

tiveness to trauma or it may possibly be a useful reaction, suited to the functions of that region which are to keep the upper part of the stomach closed, and to press the gastric contents steadily into the antral mill.

It was rather surprising to find that the latent period of smooth muscle from the antrum of the frog's stomach was often shorter than that of muscle from the antrum of the rabbit, cat and dog (the stimulus, of course, being the same). This is the more surprising in that the frog strips were tested at 19°C., the others at 37°C. Another surprise was that the frog strips did not keep so well in the ice box (in cold blooded Ringer's solution) as did those from the dog.

SUMMARY

Marked differences have been found in the reactions of muscle strips from different parts of the stomach of the frog, stimulated in a moist chamber with the faradic current.

The cardiac strips showed by far the greatest rhythmicity. They seemed to suffer most from the trauma of excision, and they recovered slowly as compared with the antral strips. They suffered more damage, also, from strong faradic currents than did the antral strips. They did not stand being kept in the ice box for twenty-four hours so well as did the strips from the antrum. The latent period of the antral strips at times was even shorter after such treatment.

The latent period almost always was longer and more variable in the cardiac than in the antral strips. In only two out of twenty-one frogs did the cardiac muscle respond more quickly than the antral.

Strips from the middle of the stomach generally resembled the cardiac ones in their behavior.

The shape of the contraction curve was different for cardiac and antral strips. The contraction and relaxation times were shorter, and the amplitude was greater in the antral strips.

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VII. DIFFERENCES IN LATENT PERIOD AND FORM OF THE CONTRACTION CURVE IN MUSCLE STRIPS FROM DIFFERENT PARTS OF THE MAMMALIAN STOMACH

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It has been shown recently that strips of muscle cut from different parts of the wall of the stomach vary markedly in their tendencies to rhythmic contraction and in the forms of the curves obtained (1). Decided differences were found also in the irritability and latent period when different parts of the stomach were stimulated with the same strength of current (2). In the accompanying paper (3), I have reviewed some of the literature on smooth muscle, and have given the reasons which led me to look for more peculiarities in the muscle strips: peculiarities that might account for the differences in rhythmicity. Work done first on the frog showed marked differences in the behavior of strips from cardia and antrum. They were kept in a moist chamber, and stimulated with the faradic current. The most striking difference was found in the forms of the contraction curves from cardia and antrum. The contraction and relaxation times were shorter, and the amplitude was greater in the antral strips. Contrary to expectation, the latent period was almost always shorter in the antral strips. A number of factors were mentioned which might account for this reversal of the conditions found when the stomach was stimulated *in situ*.

This paper deals with similar differences observed in the reactions of strips from different parts of the mammalian stomach.

TECHNIC

The apparatus and method have been described in the previous paper. The mammalian strips were kept at 38°C. The strips were obtained in the same way and from the same locations as described in Communication IV. They were cut longitudinally from the two curvatures. The sequence in which they were tested was changed daily, although this factor did not seem to have any influence on the results. The stomach was kept in the icebox, in a moist chamber, or in Ringer's solution at 6°C., and taken out from time to time as strips were cut and tested. The strips were all about 2 cm. long. The only ones that had the mucous membrane attached were the first two from the lesser curvature of the rabbit. As explained in Paper IV, a separation was not possible in this region.

Many experiments were done at first to make sure that the results obtained could be duplicated, and that they were not accidental. It was found that with the coil at 4 cm. a series of tests on the same strip would generally show about the same latent period. For instance, a strip from the middle of the greater curvature in a dog reacted after 0.29, 0.28, 0.27 and 0.27 seconds, in successive tests at intervals of fifteen minutes. Two hours later, another strip was cut from the same region, and it reacted after 0.27 seconds. As soon as it seemed certain that single observations could be trusted, the following technic was adopted. The strips were left ten minutes in the moist chamber, that they might warm up and recover somewhat from the trauma of attachment. They were stimulated then with the coil at 6 cm. A minute after they had regained their original lengths, they were stimulated with the coil at 4 cm. Again, after waiting for relaxation, they were stimulated with the coil at 0 cm. The contractions were not quite maximal with the coil at 4 cm. When the result with C.D. (Coil Distance) 4 cm. was shorter than that with C.D. 6 cm., and a little longer than that obtained with C.D. 0 cm., it was pretty sure to be correct. Most of the discrepancies met with were due probably to the difficulties in marking the record when the lever had risen very gradually at first. The mammalian strips have been much more satisfactory to work with, however, than were the frog strips; the base line has been more constant, and in most of the records the latent period could be measured accurately. The great rhythmicity which was so troublesome while studying the frog's cardia was seldom met with while working with the rabbits' stomachs, and then only with the cardiac strip. It was more disturbing in some strips from the stomachs of cats and dogs.

LATENT PERIOD

Figures 1 and 2 represent the latent periods obtained in strips from different regions of the rabbit's stomach with the secondary coil at

6 cm. and at 4 cm. The ordinates represent fractions of seconds. The lines connect readings from the several strips from one stomach. The little diagrams above show the locations from which the different strips were taken. When a number of tests were made on the same strip, the shortest latent period observed was taken for these charts. It will be seen that with the weaker current, the shortest latent periods were observed in the two strips from the cardia and from the fundus. Many strips failed to respond at all. This was true particularly of the strips from the antrum and from the middle and preantral regions of the greater curvature. The cardiac strip also failed to react in a number of animals. The fundus showed the smallest percentage of failures, while the strip from the antrum on the greater curvature responded but once, and then doubtfully.

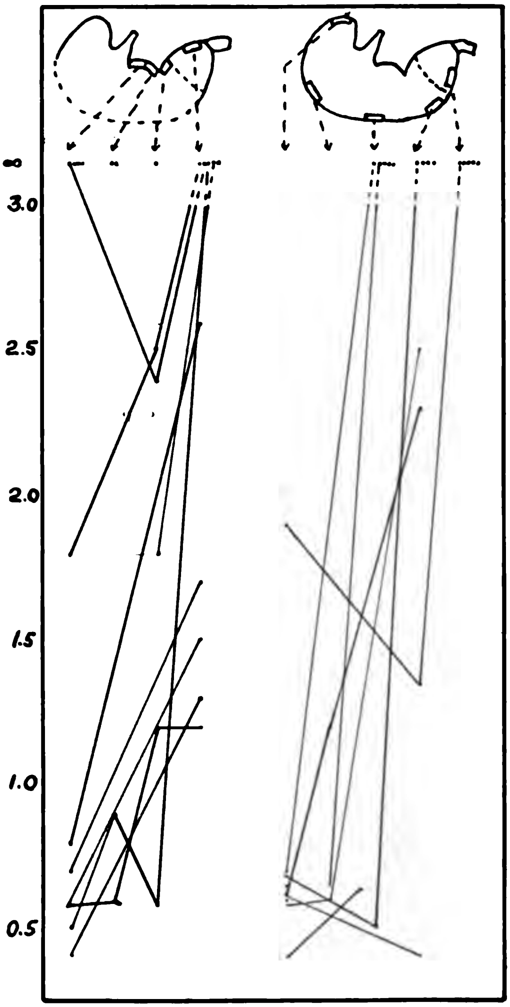

Fig. 1. Latent periods of muscle strips from the rabbit's stomach with the coil at 6 cm. The diagrams indicate the regions from which the different strips were taken. Ordinates represent seconds. The lines connect readings on strips from the same stomach.

With the stronger current, figure 2, reactions were always obtained in normal animals. Again, it will be seen that the latent period was shortest at the cardia and at the fundus. After excluding a few widely divergent results and those obtained in animals which were found to be diseased, the averages (for the rabbit) were as follows:

Lesser curvature	*seconds*
Near cardia	0.22
Middle	0.37
Preantrum	0.35
Antrum	0.65

Greater curvature	*seconds*
Fundus	0.25
Opposite cardia	0.35
Middle	0.40
Preantrum	0.41
Antrum	0.76

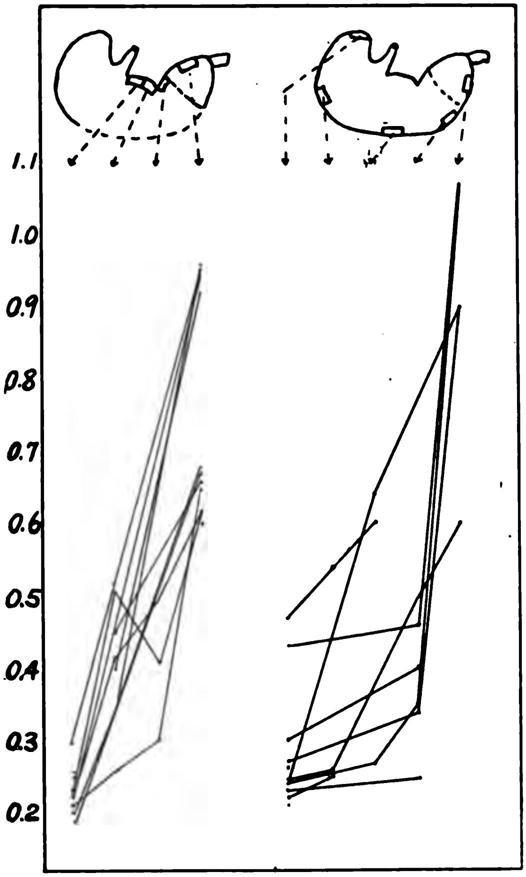

Fig. 2. This corresponds in every way to figure 1, except that the strips were stimulated this time with the coil at 4 cm.

From this we can say that, roughly, the latent period of the strips varied inversely as their distance from the cardiac end of the stomach. The latent periods of the antral strips were three times as long as those of strips from cardia and fundus.

Similar results were obtained with strips from the stomachs of a few dogs. Figure 3 shows the gradation in the latent periods of strips from different regions. Again, it will be seen that the strip from the lesser curvature next to the cardia responded usually in the shortest time, and that there is more or less gradation towards the antrum. Sometimes the latent period was shorter in the strip next to the pylorus than in the one just above in the antrum. This is in agreement with the finding in the intact stomach that the pyloric ring is more irritable than any other part of the antrum.

Observations on strips from the stomachs of a few cats indicate that the latent periods are very similar to those in the dog.

A little work was done with circular stomach strips in dogs and rabbits, but it was not satisfactory on account of the poor amplitude of the contractions obtained. This made it hard to say just where the lever began to rise. As nearly as could be determined, there was a gradation from cardia to pylorus similar to that found with the longitudinal strips.

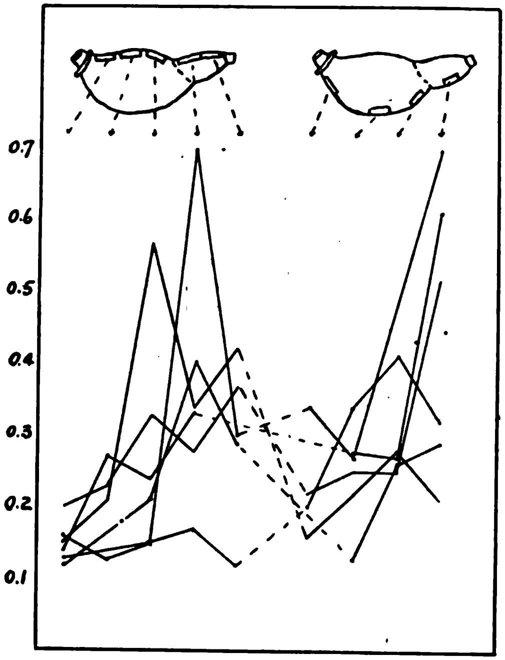

Fig. 3. Latent periods of muscle strips from the dog's stomach with the coil at 4 cm. The diagrams indicate the regions from which the different strips were taken. Ordinates represent fractions of seconds. The lines connect readings on strips from the same stomach. The lowest line on the left was from a dog that had recently been inoculated against distemper with a live culture of B. bronchisepticus. As far as could be ascertained, the other dogs were in good condition.

THRESHOLD OF IRRITABILITY

In the rabbit, the threshold was usually about the same for the four strips on the lesser curvature and for the upper two on the greater curvature. They all reacted with the coil at 6 cm. In a few animals, the cardiac and fundus strips reacted with the coil at 8 cm. In a few dogs, the strip from the lesser curvature near the cardia responded well with the coil at 10 cm., while the other strips responded only at 7 cm. and 6 cm. This was to be expected from the results on the intact stomach.

SHAPE OF THE CONTRACTION CURVE

Here, again, differences have been found similar to those already described for the frog. They perhaps can best be illustrated by the following data from the rabbit. The figures indicate seconds elapsed after stimulation. The coil was at 4 cm. Sample tracings are shown in figure 4.

	LATENT PERIOD	APEX	RELAXATION
			Cardia, lesser curvature
			seconds
	0.20	5.6	30
	0.30	5.6	31
	0.23	5.0	17
	0.25	5.0	40
	0.25	5.0	45
	0.25	6.4	45
	0.21	5.2	35
Average...	0.24	5.4	35
			Fundus, greater curvature
	0.23	13.0	After 180 seconds it had relaxed one-third.
	0.21	12.4	After 120 seconds it had relaxed very little.
	0.47	10.6	After 120 seconds it had relaxed very little.
	0.34	17.0	After 14 minutes it had relaxed very little.
	0.37	56.0	After 10 minutes relaxation was not complete.
	0.43	15.0	After 10 minutes relaxation was not complete.
	0.27	30.0	After 10 minutes it had relaxed very little.
	0.24	13.0	After 5 minutes it had relaxed very little.
	0.24	12.0	After 120 seconds it had relaxed very little.
Average...	0.31	20.0	?
			Antrum, lesser curvature
			seconds
	0.68	5.2	15
	0.95	4.7	18
	1.00	4.6	14
	0.95	4.2	16
	1.50	4.3	19
	0.65	4.0	17
Average...	0.95	4.5	16

	LATENT PERIOD	APEX	RELAXATION
			Preantrum, greater curvature
	0.25	7.4	After 50 seconds it had relaxed two-thirds.
	0.46	6.3	After 60 seconds it had relaxed completely.
	0.34	6.0	After 31 seconds it had relaxed completely.
	0.36	5.5	After 27 seconds it had relaxed completely.

Enough data have not been secured from other parts of the stomach to give averages of any value. The figures from cardia, fundus and antrum show clearly enough that there were differences in the shape of the curve sufficiently characteristic, if need had been, to have identified the different strips.

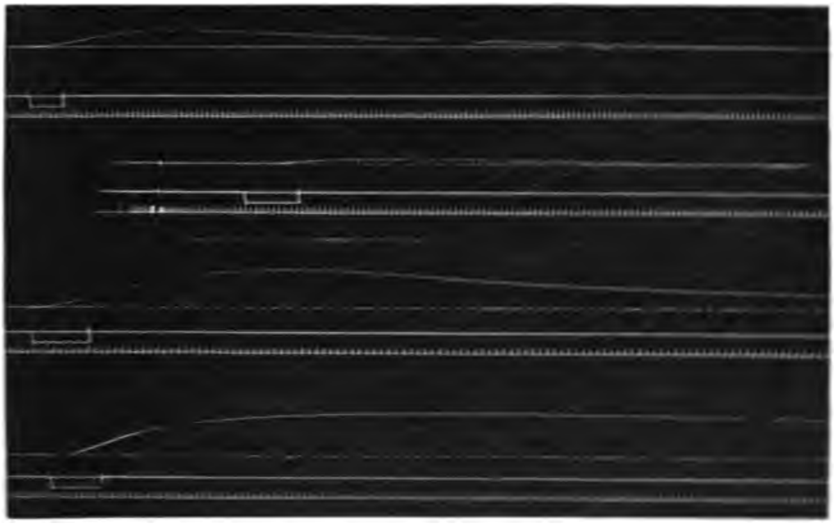

Fig. 4. Sample tracings from four strips from the rabbit's stomach stimulated with the coil at 4 cm. From above downward, they are from (*1*) the lesser curvature near the cardia; (*2*) antrum on the lesser curvature; (*3*) greater curvature just above the antrum, and (*4*) top of fundus. The magnification was the same for all. The time record represents seconds.

The antrum had the shortest contraction and relaxation times of any part of the stomach. It is strange that, with these, it should have had the longest latent period. One can not help thinking of the work of Lee, Guenther and Meleney (4) who showed that while the diaphragm excels the other skeletal muscles in almost every way, it falls behind them as regards its latent period. I have already suggested (2) that the muscle in the antrum may be different from that in the rest of the stomach, just as the diaphragm is different from other muscles, and for the same reason: because it works so constantly day and night.

The sustained contraction of the muscle from the fundus may be suited to its function. I have commented elsewhere on the fact that when strips from the fundus were put into warm aerated Ringer's solution, they gradually contracted down until they lost all tendency to rhythmic movement. This may have been due to a large contraction remainder.

The amplitude varied in the different strips. This can be seen in figure 4. In the rabbit, the antrum generally had the smallest amplitude, the fundus the greatest. The figures in the following table were obtained while using the same strength of current and the same lever magnification—4.5: 20.5. As stated before, the strips were cut with as nearly as possible the same length. The distance is given in millimeters from the base line to the highest point on the curve.

RABBIT	CARDIA	ANTRUM	FUNDUS
1	2.0	1.0	5.0
2	4.0	1.0	37.0
3	2.5	1.0	42.0
4	2.0	1.0	8.0
5	4.0	1.0	7.0
6	4.0	1.5	22.0
7	8.5	1.5	30.0

The figures for the fundus would have been even larger if the strips had not already, as a rule, been shortened by previous stimulation. It is hard to say why the amplitude was so poor in the antral strips. It was very good in the frog's antrum.

Differences were found in the shape of the curves also while studying the stomachs of dogs and cats. On the whole, the curves from the dog's stomach resembled those from the corresponding regions in the rabbit, but there were wider individual variations. Even the same strip would at times show strange differences, due possibly to changes in tone. For instance, a strip from the greater curvature reacted as follows:

LATENT PERIOD	APEX	RELAXATION
seconds	*seconds*	*seconds*
0.29	10	35
AFTER A FEW MINUTES		
0.28	38	500

Sometimes the shorter curve would follow the longer. A rapid return of the lever to the original base line might at times be due to a progressive fall in tone; occasionally it seemed due to the low amplitude, that is, the lever did not have far to fall.

Strips from the antrum of the dog generally showed a bigger amplitude than did those from the rabbit.

EXPERIMENTS ON THE HUMAN STOMACH

Through the kindness of Dr. L. L. Stanley, I was able to get strips from a normal human stomach at an autopsy held half an hour after death by hanging. Death was instantaneous; the man was middle aged, powerfully built and healthy. His stomach was full of food. The strips were cut about 10.30 a.m., strung on a small safety pin in known order, and dropped into Ringer's solution previously cooled to about 6°C. The laboratory could not be reached until 7 p.m., when the temperature in the thermos bottle was 13°C. The tests were made between 7.30 and 11 p.m.

The differences in latent period will be seen in the following table. The figures indicate seconds.

COIL DISTANCE	LESSER CURVATURE			GREATER CURVATURE		
	Cardia	Preantrum	Antrum	Fundus	Middle	Preantrum
cm.						
8	2.90	∞	∞	∞	∞	∞
6	0.44	0.68	0.70	0.45	0.55	0.85
4	0.19	0.27	0.28	0.22	0.32	0.26

These figures show a gradation from cardia to pylorus similar to that found in the rabbit, dog and cat. It is interesting, also, that the threshold was lowest in the cardiac strip. This strip was also the only one which showed enough rhythmicity to interfere with making the tests. The peculiar characteristics of the cardiac strip might have been even more pronounced if, in this case, it had not been taken from a point considerably to one side of the median line. This was done in order that the area heretofore found so interesting might be studied histologically under ideal conditions.

The following figures show the differences in the shape of the curves from different regions. They denote seconds elapsed after stimulation. Relaxation was often so slow after a certain point that I could not afford to wait for its completion.

COIL DISTANCE	LATENT PERIOD	APEX	RELAXATION
Cardia, lesser curvature			
cm.			
6	0.62	12.3	After 100 seconds little change took place.
4	0.19	25.0	After 100 seconds little change took place. Relaxation was two-thirds accomplished.
Antrum, lesser curvature			
6	0.85	5.7	After 20 seconds relaxation was complete.
4	0.28	11.0	After 30 seconds relaxation nearly complete. It was complete in 60 seconds.
Fundus, greater curvature			
6	0.45	6.5	After 60 seconds relaxation was complete.
4	0.22	6.6	After 300 seconds relaxation was two-thirds accomplished.
Middle, greater curvature			
6	0.60	8.3	After 22 seconds relaxation was complete.
4	0.32	10.0	After 300 seconds relaxation was five-sixths accomplished.
Preantrum, greater curvature			
6	0.85	9.3	?
4	0.26	60.0	After 300 seconds relaxation was six-sevenths accomplished.

No conclusions can be drawn from single experiments. It is interesting, however, to note that the antral strip relaxed promptly, as it did in the rabbit and dog.

THE LATENT PERIOD AFTER FORTY-EIGHT HOURS

While studying strips from the frog's stomach, it was found that those from the cardia did not keep well in the icebox, and their latent periods always were lengthened. Those from the antrum, however, would sometimes have a shorter latent period after twenty-four hours than they did at first. This peculiarity in the behavior of strips from the pyloric region was even more marked in the dog, where it could be observed after forty-eight hours. Between tests, the strips were kept in Ringer-Locke's solution at 6°C. The following figures show that while the forty-eight hour interval caused the cardiac latent period to lengthen 30, 136 and even 500 per cent, that of the antral strip was usually shortened; in one animal, by 75 per cent.

	CARDIA LESSER CURVATURE	ANTRUM LESSER CURVATURE	MIDDLE, GREATER CURVATURE
Dog 1			
C. D. 4 cm	0.16	0.70	0.27
After 48 hours	0.27	0.18	0.60
Percentage	168	25	222
Dog 2			
C. D. 4 cm	0.22	0.35	0.29
After 48 hours	0.52	0.31	0.70
Percentage	236	88	241
Dog 3			
C. D. 4 cm	0.24	0.31	0.63
After 48 hours	0.35	0.34	1.10
Percentage	146	110	174
Rabbit			
C. D. 4 cm	0.20	0.68	0.50
After 24 hours	1.20	0.40	∞
Percentage	600	58	
Cat			
C. D. 4 cm	0.30	0.85	0.31
After 24 hours	0.39	0.30	0.32
Percentage	130	35	103

It would seem as if the antral muscle were not only much more resistant to the conditions that bring about death, but that it must, during the 48 hours, get rid of some form of inhibition.

DIFFERENCES IN DISEASED ANIMALS

Before the work had progressed far, it was noticed that, with certain stomachs, all but one or two of the strips would react poorly, the irritability would be low, the latent period longer than usual, and the amplitude small. These strips did not keep well in the icebox, and after twenty-four hours, might not react at all to strong stimuli. The following few figures from the rabbit show that, compared with the averages for healthy animals, the strips from the fundus and middle of the greater curvature did not suffer at all. Those from the lesser curvature, however, suffered a good deal.

Rabbit

	LESSER CURVATURE			GREATER CURVATURE				
	Cardia	Prean-trum	Antrum	Fundus	Opp. Cardia	Middle	Prean-trum	Antrum
March 15, 1915..........	0.85		1.00	0.21		∞		
December 6, 1915..........	0.60	0.90	1.40	0.22	0.25	0.50		0.60
April 3, 1916..........	1.05		1.50	0.27				
July 8, 1916..........	0.70	1.15	1.50		1.43	0.27	1.65	∞
Normal average...........	0.22	0.35	0.65	0.25	0.35	0.40	0.41	0.76

C. D. was 4 cm. in all.

The first animal seemed to have some intestinal disturbance as the bowel was distended and flabby; the second had snuffles; the third had large, inflamed Peyer's patches and some indurated areas in the cecum, due apparently to encysted parasites; while the fourth had recently had an abortion, judging from the appearance of the uterus.

Similar deviations from normal were found in strips from the stomachs of distempered dogs. There, the most marked feature was the great shortening of the latent period in the strips from the pyloric antrum. In two dogs, this shortening caused an actual reversal of the gradient, the antral strip contracting before the cardiac one. The figures obtained on successive tests with the coil at 6 cm. (4 cm. unfortunately was not used) are as follows:

	Lesser curvature	*Lesser curvature*
	Cardia	*Antrum*
Small male spitz.	0.40; 0.45; 0.40; 0.39	0.22; 0.30; 0.30
Mongrel female.......	0.85; 0.90; 0.90	0.65; 0.60; 0.65; 0.70; 0.70

It will be noted, in figure 3, that one of the lines is unusually low, and that the antrum responded more promptly than the cardia. These records were included in the chart because the dog seemed healthy and normal in every way. Inquiry showed later, however, that that dog had recently been inoculated against distemper with a live culture of *Bacillus bronchisepticus*. It seems probable, then, that the reversal of the gradient on the lesser curvature was due to this infection.

The following figures are from cardia and antrum of a large old mongrel dog which had been vomiting for several days. He was *in extremis* when killed by ether and bleeding. Autopsy showed a severe chronic nephritis. One kidney contained a stone, and the prostate was markedly hypertrophied. The lungs were normal.

	Lesser curvature *Cardia*	*Lesser curvature* *Antrum*
C. D. 8 cm.	∞	∞
C. D. 6 cm.	0.57	∞
C. D. 4 cm.	0.19	0.18
C. D. 0 cm.	0.22	0.17

It will be seen that although the irritability was greater in the cardiac strip, the latent periods with strong stimuli were shorter in the antrum. The reversal of the gradient was due mainly to the marked shortening of the antral latent period. A similar reversal was found in the stomachs of two monkeys killed in the last stages of dysentery.

DISCUSSION

The gradation in the latent period from cardia to pylorus on both curvatures agrees with the gradation of rhythmicity previously described. This work has emphasized again the peculiar properties of the muscle in the cardiac region on the lesser curvature, and has made it seem more likely that that is a rhythmic center for the stomach. There was some question at first whether or not there might be another such center in the preantral region on the greater curvature. The waves often seem to arise there, and strips from that area generally showed considerable rhythmicity. This work indicates now that the muscle there is not very different from that in the areas near by. Certainly it does not stand out as peculiarly as the cardiac muscle does.

It is possible that the greater irritability and shorter latent period in the lower than in the upper part of the antrum might tend to stop the waves as they reach the pylorus. It is well known that the waves do not sweep over into the duodenum. Often in the rabbit I have seen, much as Auer (5) has described, a small contraction appear at the pylorus and, moving upwards, break against the down-coming wave in the lower antrum. The process makes one think of backfiring in a forest. This might occur, perhaps, if the shorter latent period in the muscle of the pyloric ring should cause it to pick up the stimulus ahead of its turn.

If the gradation of latent period, irritability, rhythmicity, etc., from cardia to antrum plays any part in the normal progress of peristalsis, it seems likely that an upset in this gradient should have important consequences. There might then be a tendency to anastalsis, the stomach would not invite food, and appetite might be lost. It must be remembered, of course, that the figures given here have been obtained from excised strips, stimulated powerfully. The usual stimuli received by the stomach are probably much weaker. Moreover, the gradient of latent period and irritability for such stimuli in the highly sensitive intact stomach is probably steeper than it is in the strips. Even in the strips, the gradient is generally steeper with weak stimuli. Thus in the table of results on the human stomach, it will be seen that with the coil at 8 cm. the gradient is from 2.90 seconds at the cardia to ∞ at the pylorus; with the coil at 4 cm. it is from 0.19 second at the cardia to 0.28 second at the pylorus. We can not say, as yet, how much the gradient can be changed in the intact stomach. Certainly it is interesting to know that the muscles in cardia and antrum are so different that the one can be slowed and the other quickened by the same agent. The subject invites further research.

SUMMARY

Differences have been found in the latent period of muscle strips removed from different parts of the stomach wall. Speaking roughly, the latent period varies inversely as the distance from the cardia.

The irritability was greatest in the strip from the lesser curvature next to the cardia.

There were differences in the shape of the contraction curve sufficiently characteristic, if need had been, to have identified a number of the different strips.

Similar results were obtained while studying strips from a human stomach.

When the strips were left 48 hours in the icebox, those from the antrum showed themselves peculiar in that their latent periods were shorter, while those of the other strips were more or less lengthened.

In diseased animals, all but one or two strips generally showed a lessened irritability and a lengthened latent period. In some animals, the latent period of the antral strip was markedly shortened. The lengthening at the cardia and the shortening at the pylorus upset the normal gradient in several animals. It seems likely that if such changes should take place in the intact stomach, digestion might be embarrassed.

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REFLEX RESPIRATORY CHANGES AND THE CONCOMITANT THRESHOLD STIMULUS

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Considerable work has been done on the ways in which respiration may be modified reflexly by stimulating cutaneous sensory nerves (1), e.g., sciatic, radial, ulnar, median and trigeminal, or by stimulating visceral sensory nerves (2), e.g., vagus and superior laryngeal. Numerous experimenters have shown that varying the strength of the stimulus in one sensory nerve, e.g., phrenic (3), vagus (4), sciatic, crural, median, ulnar (5) or trigeminal (nasal branch) (6) may modify respiration.

Rosenthal (7) and later Meltzer (4) measured the strength of stimulus (in mm. the distance between the secondary and primary coils) necessary to bring about complete cessation of respiration in rabbits upon stimulating the central end of the cut vagus nerve. Rosenthal obtained only inspiratory inhibition of respiration but Meltzer obtained inhibition of respiration in both inspiration and expiration in the same animal upon vagus stimulation.

Bidder (8) and Blumberg (9) found, in cats, that the strength of current necessary to stop respiration in expiration upon stimulating the central end of the superior laryngeal nerve was weaker than that necessary to stop respiration in inspiration upon stimulating the vagus.

Nice (10) recently employed the Martin method (11) in studying the strength of stimulus necessary to alter respiration.

An attempt has been made in this article to answer the following three questions:

1. What are the average threshold stimuli of the vagi, superior laryngeal and cutaneous sensory nerves (such as the popliteal, peroneal, radial, ulnar and median) which bring about any change in the depth of respiration?

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[1] Part of the data in this research was collected from the experiments performed upon animals in which the effect of varying the rate of stimulation upon the vasomotor centre was studied. These experiments were performed in the laboratory of Physiology in the University of Pennsylvania.

2. What average strength of current is necessary to bring about certain respiratory changes (expiratory and inspiratory decrease and complete inhibition in both phases) upon stimulating the central end of the vagus nerve?
3. What effects do different rates of stimuli have upon the respiratory centre and the threshold stimulus?

THE METHOD

These experiments were performed upon cats narcotized with urethane (2 grams per kilo by stomach) and tracheotomized. Respiratory movements were recorded by catching a small S-shaped hook in the left side of the diaphragm about midway between the central tendon and the lateral chest wall. From this hook a thread was passed over a pulley to a writing lever which wrote on a kymograph surface. The vagi were then exposed and cut and the electrodes placed centrally to the point of severance. No attempt was made to isolate the inferior laryngeal branch. After the determinations for the vagi were completed readings were made whenever possible on the following nerves in the order named; superior laryngeal, peroneal, popliteal, radial, ulnar and median nerves. Stimuli were applied by means of platinum electrodes. The nerves undergoing stimulation were protected by glass shields like those described by Sherrington (12).

The current. A glass knife blade key run by a motor made and broke the primary circuit from one to twenty times per second when the effects for changes of rate were being studied, and ten times per second for determining the effects of changes of strength of stimuli. The threshold stimulus was measured in the β units of the Martin method—Z units were also used for purposes of comparison in cases where the readings were made upon the same nerve. For these readings the electrodes were not moved after they were once in place and the tissue resistance therefore remained the same.

The strength of the current in the primary circuit was 0.1 ampere for all nerves except the superior laryngeal in which a current of 0.05 ampere was employed.

RESULTS

In 16 experiments there was a decrease in the depth of inspiration with an apparently normal depth of expiration (see fig. 1). In this figure at (*1*) the strength of the stimulus was 4.2, at (*2*) 4.6 and at

(3) 5 Z units. The average threshold for the whole series of experiments was 3.2 Z units and 2 β units (see table 1). To bring about the same changes upon stimulating the left vagus the average threshold stimulus was, for 10 experiments, 3.3 Z and 1.9 β units. In my experiments I did not find it necessary to leave one vagus intact as did Nice (13). In the early experiments, threshold readings were made on one vagus both before and after cutting the other. The threshold remained the same, so later both vagi were used for making readings. Nice (10) obtained slightly higher readings 2.87 and 2.52 β units and 4.15 and 4.59 Z units probably because he used slower rates of stimulation, 4 and 6 stimuli per second. Table 2 and figs. 4 and 5 are given here to show that this change in the rate of stimulation could be responsible for the increase in the threshold stimuli in Nice's experiments. In this table I used three rates of stimulation, 4 interruptions per second, at which rate the average threshold was 4.2 Z units, 10 per second with a threshold of 3.5 Z units and 20 interruptions per second with a threshold of 3 Z units. As can be seen Nice's 4.12 Z units and my readings 4.2 Z units taken at the same rate of stimulation—4 per second—are the same, considering this method accurate only within 1 or 2 per cent. This change in respiration, the production of a decrease in the depth of respiration at the expense of the inspiratory phase, is similar to the changes described by Rosenthal (14). Knoll also observed that the inspiratory movements were not as deep when the vagus was stimulated by its own demarcation current (15).

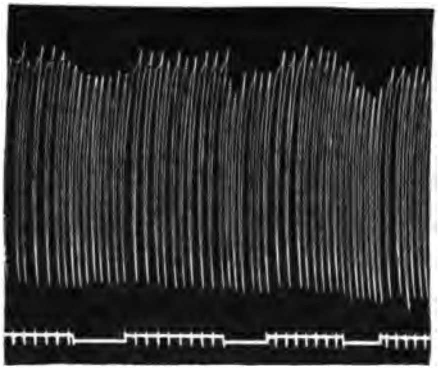

Fig. 1. Upper record tracing of respiratory movements. Lower line time in 5 second intervals and the time of stimulation. Rate of stimulation 10 interruptions per second (explained in text). In this and following curves the up stroke in the respiratory curve is inspiration, the down stroke expiration.

The superior laryngeal nerves do not require as strong a current to bring about the same response. For the right nerve the average threshold stimulus for 14 experiments was 1.5 Z and 0.8 β units and for the left the average threshold stimulus for 10 experiments was 1.3 Z and 0.7 β units (see table 1).

TABLE 1

The relative threshold stimuli (Martin Z and β units) necessary to alter the respiratory movements upon stimulating the left and right vagi and superior laryngeal nerves in the cat in urethane narcosis. Rate of stimulation ten times per second

RIGHT VAGUS				LEFT VAGUS				RIGHT SUPERIOR LARYNGEAL		LEFT SUPERIOR LARYNGEAL	
Decreased inspiration with normal expiration		Increased inspiration with decreased expiration		Decreased inspiration with normal expiration		Increased inspiration with decreased expiration		Decreased depth of inspiration with normal expiration		Decreased depth of inspiration with normal expiration	
Z	β	Z	β	Z	β	Z	β	Z	β	Z	β
1.6	0.8	2.5	1.6	2.9	2.2	5.3	4.0	1.2	0.9	1.2	0.8
2.0	1.3	4.5	3.0	1.6	0.9	4.7	3.1	1.0	0.3	1.6	1.0
2.7	1.7	5.1	3.2	3.3	1.5	6.6	3.1	1.6	1.0	1.2	0.5
3.2	1.6	7.5	3.8	3.8	2.4	5.7	3.5	1.8	0.5	1.3	0.4
5.8	3.4	7.7	4.5	2.7	1.0	6.1	2.4	1.0	0.4	1.0	0.6
6.5	4.8	13.1	9.8	4.5	2.9	11.8	8.2	1.0	0.9	1.1	0.7
1.5	1.0	2.6	1.6					1.6	0.7	1.7	0.4
0.8	0.5	2.4	1.5					1.4	0.8	1.3	0.8
1.8	1.1	4.7	3.0					1.1	0.6	1.2	0.7
2.7	1.6	5.8	3.7					1.7	1.0	1.4	0.9
2.7	1.7	4.3	2.8					1.3	0.8		
4.2	3.3			3.7	1.8			1.2	0.9		
3.3	1.9			3.3	1.9			1.6	0.8		
4.9	3.1			3.9	2.8			3.0	1.9		
3.4	1.7	5.0	2.5								
3.6	2.5										
		4.7	4.0	2.9	1.6						
Average.....3.2	2.0	5.4	3.5	3.3	1.9	6.7	4.1	1.5	0.8	1.3	0.7

TABLE 2

The threshold stimulus of the vagus as affected by changing the rate of interrruption of the stimulating current

RATE OF STIMULATION 4 PER SECOND	RATE OF STIMULATION 10 PER SECOND	RATE OF STIMULATION 20 PER SECOND
Z units	Z units	Z units
2.9	2.6	2.3
3.2	2.6	2.3
5.0	4.6	3.2
4.2	2.8	2.2
3.5	3.2	1.7
3.2	2.6	2.2
4.0	3.4	3.2
8.5	6.5	5.7
3.8	3.7	4.0
3.4	3.2	3.1
4.2	3.5	3.0

Aside from the phenomenon just described, three other effects were produced by stimulation of the central end of the cut vagus.

With an average current strength of about 6 Z units or about 4.0 β units the expiratory phase of respiration is decreased. The recording lever, therefore, does not return to the base line (see fig. 2). Here at (*1*) the strength of stimulus was 5.7 at (*2*) 6.5 and at (*3*) 7.5 Z units. In this figure there is observed upon the cessation of stimulation an inhibition of respiration in inspiration.

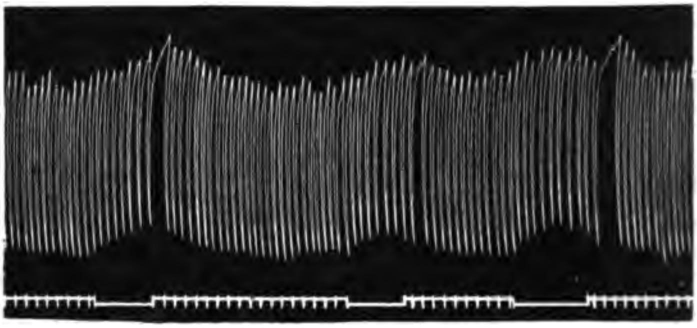

Fig. 2. Upper curve respirations. Lower line time in 5 second intervals and time of stimulations (explained in text).

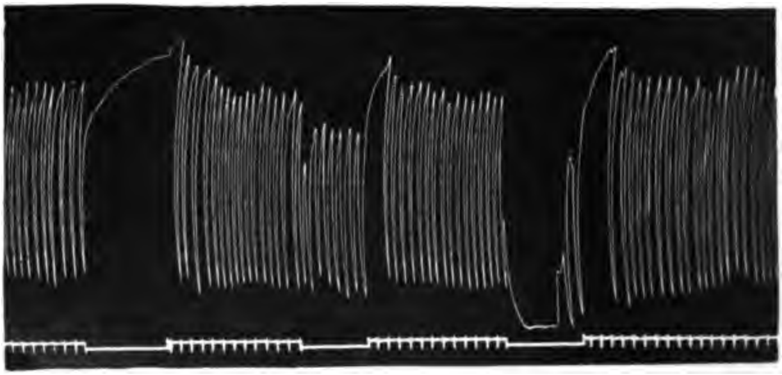

Fig. 3. Upper curve respiration. Lower line time in 5 second intervals and time of stimulation. At (*1*) the strength of the stimulus was 25 Z units, at (*2*) 59 Z units, and at (*3*) 175.5 Z units.

As the strength of the current is further increased with a uniform rate of stimulation—10 per second—a point is reached at which respiration is inhibited in inspiration. As is almost always the case considerable variation is observed in the strength of current necessary to bring about these changes in different animals. Meltzer (4) observed this same variation in his experiments upon the vagus nerve. If the nerve is not fatigued, he says, it is possible to bring about the same changes in respiration repeatedly in the same animal with the same strength of current (p. 351).

TABLE 3

The threshold stimulus of cutaneous sensory nerves as measured by the Martin method

NERVE STIMULATED	Z	β	AVERAGE Z	AVERAGE β
Popliteal	15.7	7.9		
	7.2	3.4		
	7.2	5.1		
	11.8	7.0		
	7.7	4.2		
	10.9	7.4		
	8.7	8.2		
	5.1	3.4	9.3	5.6
Peroneal	5.8	3.1		
	10.0	4.3		
	6.7	4.5		
	6.7	3.8		
	10.0	8.2		
	10.9	7.9	8.3	5.3
Radial	5.1	4.2		
	3.7	2.6		
	8.2	6.2		
	8.7	4.2		
	11.9	5.0	7.5	4.4
Ulnar	5.5	3.0		
	9.4	6.0		
	7.7	4.2	7.5	4.4
Median	6.6	4.1		
	11.5	9.1		
	9.4	6.5	9.4	6.6
Average	8.5	5.3	8.4	5.3

In this series of experiments inhibition of respiration in inspiration was produced with 61 Z units as an average (see fig. 3). Such a reaction was obtained in twenty-six out of twenty-nine animals experimented upon.

With a still further increase in the strength of current it was possible to bring about a cessation of respiration in expiration. The average strength of current required was 299 Z units. Thirty-one of thirty-three animals gave this response (see fig. 3). In many cases it was possible also to demonstrate this in another way. If, with a strength of stimulus sufficient to bring about inhibition of respiration in inspiration at 4 stimuli per second, the number of stimuli was increased to 20 per second the response was an inhibition of respiration in expiration (see fig. 5).

Rosenthal believed that such an expiratory inhibition was due to an escape of current to the superior laryngeal nerve. Meltzer did not agree with Rosenthal and demonstrated that respiration could be stopped by a strong stimulation of the vagus. I found that if a silk ligature was tied very tightly around the vagus central to the point of stimulation, no change in respiration resulted upon stimulation although previously a complete inhibition of respiration was brought about with the same strength and rate of stimulation.

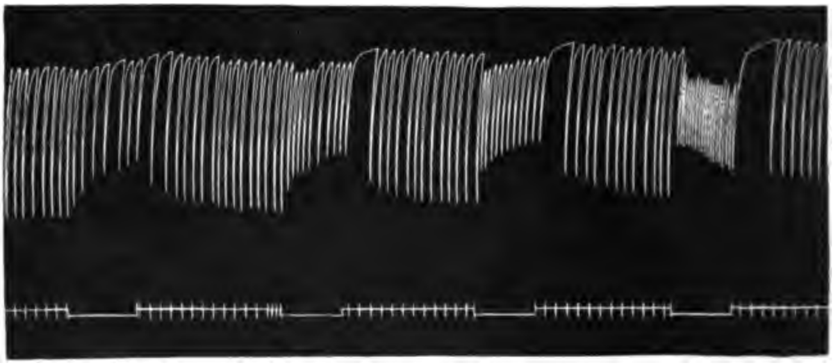

Fig. 4. Upper curve respiratory movements lower curve time in 5 second intervals and time of stimulation. Strength of stimulus 16.8 Z units. Rate of interruption, at (*1*) 4, (*2*) 7, (*3*) 10, (*4*) 20 per second.

A current of only 19.5 Z units was necessary to stop respiration in expiration upon stimulating the superior laryngeal nerve. This average was taken from readings collected from 14 experiments.

The readings upon the cutaneous sensory nerves (peroneal, popliteal, median, radial and ulnar) are considered together here. For the 25 readings made upon them the average threshold stimulus for the respiratory reflex was 8.5 Z units and 5.3 β units (see table 3).

I did not find as did Meltzer (15) that the sex of the animal experimented upon affected the response obtained upon vagus stimulation. In only five out of thirty-four animals was it impossible to obtain both inspiratory and expiratory inhibition. It was, however, very difficult and frequently impossible to bring about a complete inhibition in inspiration after inhibition in expiration had been produced.

Figures 4 and 5 are given here to show how the rate of stimulation affects the respiratory centre. In figure 4 the strength of stimulus was 16.8 Z units with a changing rate. At (*1*) the rate was 4 per second. There resulted a decrease in both the rate and depth of respiration. At (*2*) the rate was 7 per second with a resulting increase in rate and decrease in the depth of respiration. At (*3*) the rate was 10 per second and at (*4*) 20 per second each accompanied by a change in the rate and depth of respiration. Figure 5 shows a still more marked change. At (*1*) the rate of stimulation was 7 per second and the strength was 220 Z units. This caused an inhibition of respiration in inspiration. At (*2*) the rate of interruption was 20 per second with the same strength of current, 220 Z units. Here respiration ceased in expiration.

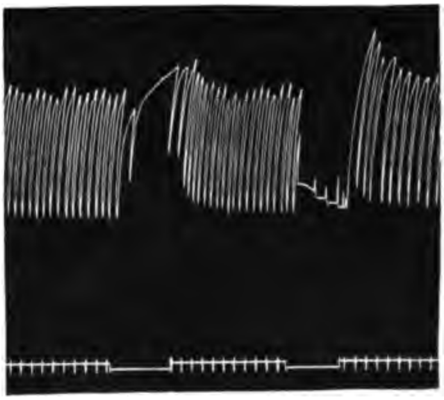

Fig. 5. Upper record respiration. Lower curve time in 5 second intervals and time of stimulation. At (*1*) rate of stimulation 7 per second. At (*2*) rate of stimulation 20 per second. Strength of stimulus 220 Z units.

Since the rate of interruption has an effect on the changes resulting from reflex excitation of the vasomotor center (16) and upon the respiratory center the question arose as to whether it might not be accountable for the difference in results obtained by Mendenhall (17) and by me upon stimulation of the cervical sympathetic (18).

Five series of threshold readings with various rates of stimuli were made using the retraction of the nictitating membrane as an index.

With a rate of 4 per second the threshold stimulus was 9 Z units, when 10 per second 7.5 and when 20 per second 5.3 Z units.

SUMMARY

1. In this series of experiments the threshold stimuli necessary to bring about a change in respiration upon stimulating the central ends of the cut vagi were 3 Z units and 2 β units for the right and 3 Z units and 1.9 β units for the left.

2. The threshold stimuli for the superior laryngeal nerves necessary to bring about a change in respiration were 1.4 Z and 0.8 β units for the right and 1.3 Z and 0.7 β units for the left. This is lower than that required by the phrenic diaphragm preparation used by Nice.

3. Sensory nerves such as the popliteal, peroneal, ulnar, radial and median nerves require a comparatively strong stimulus to effect respiration. The average stimulus was 8.3 Z and 5.2 β units.

4. A slow rate of interruption and strong stimuli have less effect upon the respiratory centre than do a high rate of interruption and a weaker stimulus.

5. The strength of stimulus required to bring about cessation of respiration in inspiration is less than that required to bring about a cessation of respiration in expiration providing the rate of stimulation remains the same. With a fixed strength of stimulus the cessation of respiration in expiration requires a faster rate of stimulation than does cessation of respiration in inspiration.

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STUDIES IN EXPERIMENTAL GLYCOSURIA. XII

LACTIC ACID PRODUCTION IN THE BLOOD FOLLOWING THE INJECTION OF ALKALINE SOLUTIONS OF DEXTROSE OR OF ALKALINE SOLUTIONS ALONE

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In the previous paper of this series (1) it was shown in confirmation of the work of other investigators (Murlin, Underhill) that addition of alkalies to the blood causes the percentage of blood-sugar to become lowered. There are three possible mechanisms concerned in the control of the blood-sugar level which the alkali may influence so as to bring about this change, namely: (1) the glycogenic function of the liver and muscles; (2) the oxidation of dextrose in the muscles, and (3) destruction of dextrose in the blood itself. Evidence was furnished in the above referred to communication that the alkali does not perceptibly affect the glycogenic function. In the present research we have examined the blood for the presence in it of possible break-down products of dextrose, and have chosen lactic acid as the most likely one to occur. The general plan of the investigation has consisted in the collection of samples of blood that were large enough to make an accurate determination of lactic acid possible, at intervals from the upper portion of the portal vein, or from the vena cava opposite the liver before and during the injection, lower down in the portal vein, either of dextrose solutions in the presence of a trace of acid or an excess of alkali (sodium carbonate), or of alkaline solutions without any dextrose.[1]

In the accompanying tables are shown the results of the experiments which may be divided into three groups: In the *first group* (Experiments I and II) the lactic acid was determined in blood drawn from the pancreatico-duodenal vein: (a) before and (b) during the injection of an alkaline solution of dextrose; (c) a few minutes after discontinuing the injection; and (d) during a subsequent injection of a similar amount of dextrose in faintly acid solution.

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[1] The details of the method used for estimating the lactic acid will be published elsewhere.

It will be noted that within fifteen minutes after starting the injection of alkaline dextrose solution the percentage of lactic acid in the portal blood increased in the first experiment from 0.056 to 0.096 per cent (71 per cent increase), and in the second, from 0.066 to 0.123 (86 per cent increase). The increase is more marked in the experiment in which the dextrose was injected in moderate amount (at the rate of 0.34 gram per kilogram and hour), but the alkali was excessive (20 per cent Na_2CO_3), than in that in which the dextrose was in excess (0.73 gram per kilogram and hour) and the alkali moderate (10 per cent Na_2CO_3). This result would indicate that the factor upon which the lactic acid production depends is the alkalinity of the dextrose solution rather than the actual amount of dextrose injected. The behavior of the portal blood-sugar further corroborates this view, a much greater increase in blood-sugar being evident in the experiment (No. I) which shows the lesser increase in lactic acid.

The percentage of lactic acid was still undiminished in blood removed in ten minutes after discontinuing the injection (Experiment II). This indicates either that the lactic acid is only slowly oxidized by the animal or that the alkali continues to produce lactic acid from the dextrose of the blood, or from that injected along with the alkali, for some time after discontinuing the injection. It is also possible that breakdown products of the action of alkali on dextrose continue to circulate in the blood for some time after the blood has regained its normal H-ion concentration, and that these only become converted into lactic acid as some of this becomes oxidized. In other words, the lactic acid after accumulating to a certain extent may limit any further production in obedience to the laws of mass action.

In both experiments, following a period during which no injection was made, dextrose was again injected, but in faintly acid solution (2 to 3 drops concentrated HCl per 100 cc. of solution). In Experiment I, although distinctly more dextrose was injected than during the previous alkali period, the lactic acid percentage was found to be about 10 per cent lower. In the light of results which we will discuss immediately it is probable that the increase which does exist in this part of the experiment would have been much less evident had a longer period of time been allowed to elapse following the injection of alkali.

In Experiment II, the lactic acid percentage during the injection of acid remained at its previous high level, a result which is in harmony with the above stated view, namely that there still remained in the blood of this animal an accumulation of lactic acid or of some precursor produced by the action of the alkali.

In the *second group* (Experiments III and IV), the dextrose was first of all injected in faintly acid or neutral solution and later in the presence of alkali.

In Experiment III a 5 per cent solution of the purest dextrose obtainable (Merck's) was injected in the usual way, first of all in faintly acid solution and later in alkaline. The rate of injection was a little quicker during the latter as compared with the former period. On account of omission to record the weight of the animal, the exact dosage per kilogram body weight cannot be given, but we know that it was well within the utilization value of 0.8 gram per kilogram an hour. Lactic acid was determined in blood removed as nearly as possible simultaneously from the portal vein and vena cava, the object of this being to see whether any of the lactic acid produced in the portal blood would be retained by the liver. The blood-sugar was determined by the Bertrand method in aliquot portions of the Schenck filtrate, the remainder of which was used for estimation of the lactic acid.

It will be noted that practically no increase in lactic acid was produced in the blood of either vessel, as a result of the injection of dextrose in acid solution,[2] whereas, on the other hand, an increase from 0.039 to 0.076 (95 per cent) occurred in the blood of the portal vein, and from 0.050 to 0.092 (85 per cent) in that of the vena cava, following the injection of the alkaline solution. The increase in dextrose-content was only moderate and approximately the same during both periods.

Two conclusions may be drawn from the results of this experiment: first, that the injection of a faintly acid solution of chemically pure dextrose does not cause any perceptible increase to occur in the lactic acid content of the blood, and secondly, that when such an increase does occur as a result of the injection of practically the same amount of pure dextrose dissolved in alkali, the lactic acid thus formed is not retained by the liver. Why there should have been somewhat more lactic acid in the blood of the vena cava than in that of the portal vein, we cannot explain.

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[2] Some loss is known to have been incurred in this estimation on account of clotting of blood during its collection.

In Experiment IV, the procedure was in general the same as that of Experiment III, except that blood for analysis was removed from the portal veing alone (an unmanageable clot having formed in the vena cava cannula). The dextrose was not so pure a specimen as that used in the previous experiment (Mulford's), and it was injected at first in neutral reaction and later in the presence of 15 per cent Na_2CO_3.

It will be noted that the lactic acid content was initially high in this animal, and that if anything it was very slightly increased (from 0.065 to 0.067 per cent) by the injection of a neutral solution of dextrose at a rate of 0.36 gram per kilogram and hour. The injection of the alkaline solution was then started at a rate which was at first only about two-thirds that of the neutral solution (0.26 gram per kilogram and hour), and yet, within ten minutes after starting the injection, by which time 3 grams Na_2CO_3 had been injected, the lactic acid percentage had increased from 0.064 to 0.074 (15 per cent increase). The injection rate was then greatly increased (to 2.5 gram dextrose per kilogram and hour, with a total injection of 6 grams Na_2CO_3), with the result that within three minutes the lactic acid percentage had mounted from 0.074 to 0.095 (29 per cent increase over previous estimation or 46 per cent over normal).

In Experiment V, dextrose was injected in large dosage (over 1 gram per kilogram and hour) into the mesenteric vein, blood for analysis being collected from the vena cava. During the first period of about 15 minutes a dextrose solution which was faintly acid in reaction caused no increase in the lactic acid percentage of the blood. In ten minutes after discontinuing the injection, however, the percentage had risen from 0.05 to 0.065. A similar amount of dextrose in sodium carbonate solution was then injected, with the result that in about ten minutes the lactic acid percentage of the blood had almost doubled (from 0.065 to 0.125). The results of this experiment corroborate those recorded above in two particulars: first, the dextrose injected in acid solution causes no increase in the lactic acid content of the blood, whereas a similar dextrose injection in alkaline solution causes a marked increase; and secondly, that the lactic acid thus produced is not retained by the liver.

Most of the above observations were made during the course of experiments which were primarily designed to study the behavior of the blood-sugar level during the injection of dextrose solutions in the presence of acid or alkali. On this account the experiments on the effect on the lactic acid content of the blood of injections of alkali alone which form the experiments of *group three* were not performed until quite recently, and from the results (Nos. VI and VII in the table) it is plain that the lactic acid is actually produced in the blood itself and is not, as we had thought probable, dependent upon the presence in the injected alkaline dextrose solutions of decomposition products. Thus, by the injection of Na_2CO_3 in 15 per cent solution at the rate of 0.6 gram per minute into a dog weighing 8.7 kilogram (Experiment VI), the lactic acid per 100 cc. blood increased, after twelve minutes' injection, from an average of 0.030 gram to 0.063 gram, and after fifteen minutes, it had risen further to 0.076 per cent. In the other experiment of this nature (No. VII) the alkali was injected a little quicker (viz., 0.66 gram per minute in a somewhat smaller dog) with the result that in six minutes the lactic acid content of the blood rose from 0.033 to 0.093, at about which level it also stood in eleven minutes.

These are of course very large amounts of alkali, but they are comparable with those of the dextrose solutions used in the previous experiments. There can be little doubt that a distinct increase in lactic acid of the blood would also be produced by the injection of much less of the carbonate, but we have not as yet determined just exactly how much must be injected in order to cause a measurable increase.

It is possible that more lactic acid would be produced by injections of dextrose plus alkali than by alkali alone. If dextrose be the source of the acid, such would be the expected result, and although the alkaline solutions of dextrose do not, on standing for a short time at room temperature, form any detectable amount of lactic acid, yet there is evidence from the "browning" of the solutions that some considerable decomposition of the dextrose molecule has set in. It may be that certain of the decomposition products are very promptly acted on by some enzyme present in blood (of the glyoxylase class) to form lactic acid.

That the addition of weak alkali to sterile blood *in vitro* increases the rate of disappearance of dextrose (2), and its addition to suspensions of leucocytes causes lactic acid to be produced from dextrose (3) are facts which have an interesting bearing on our results.

There are many interesting phases of the problem which await further investigation. We have placed the above results on record at the present time because of the bearing which they have in connection with the influence of alkali injections on blood-sugar.

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TABLE

NO. AND GENERAL NATURE OF EXPERIMENT	WEIGHT OF DOG	SUBSTANCE BEING INJECTED INTO MESENTERIC VEIN DURING PERIOD	DURATION OF PERIOD	VESSEL FROM WHICH BLOOD COLLECTED FOR ESTIMATION	DEXTROSE IN BLOOD (AVERAGE OF SEVERAL SAMPLES COLLECTED DURING PERIOD)	LACTIC ACID (IN SAMPLES COLLECTED DURING PERIOD)	PERCENTILE CHANGE FROM NORMAL
	kg.		*min.*				
I. Alkaline, then faintly acid dextrose solution injected	13.4	*a.* No injection		Pancreatico duodenal	0.115	0.056	
		b. 0.73 gram dextrose per kilogram, hour, *plus* 3.2 gram Na_2CO_3 in 10 % solution	15	Pancreatico duodenal	0.195	0.096	71 % increase
		c. No injection	15	Pancreatico duodenal	0.137		
		d. 1.07 gram dextrose per kilogram hour, *plus* trace of HCl	10		0.195	0.87	55 % increase
II. Alkaline, then faintly acid dextrose solution injected	20	*a.* No injection		Pancreatico duodenal	0.078	0.066	
		b. 0.34 gram dextrose, (in 4 % solution) per kilogram, hour, *plus* 8 gram Na_2CO_3 in 20 % solution	14	Pancreatico duodenal	0.110	0.123	86 % increase
		c. No injection	10	Pancreatico duodenal	0.098	0.121	83 % increase
		d. 0.32 gram, dextrose (in 4 % solution) per kilogram, hour, *plus* trace HCl	10	Pancreatico duodenal	0.149	0.125	89 % increase

III. Faintly acid, then alkaline dextrose solution injected		*a*. No injection		Pancreatico duodenal	0.119	0.039	
				Vena cava	0.093	0.050	
		b. 0.085 gram dextrose per minute in faintly acid solution*	10	Pancreatico duodenal	0.131	0.043	11 % increase
				Vena cava	0.152	0.041	18 % increase
		c. 0.10 gram dextrose per minute *plus* 2.8 gram Na_2CO_3 in 10 % solution†	13	Pancreatico duodenal	0.146	0.076	95 % increase
				Vena cava		0.092	85 % increase
IV. Neutral, then alkaline solution injected	26.9	*a*. No injection		Pancreatico duodenal	0.087	0.065	
				Vena cava		0.062	
		b. 0.36 gram per kilogram hour in neutral solution	22	Pancreatico duodenal	0.167	0.067	3 % increase
			25	Pancreatico duodenal		0.064	
		c. 0.26 gram dextrose per kilogram, hour, *plus* 3 gram Na_2CO_3 in 15 % solution	9	Pancreatico duodenal		0.074	15 % increase
		d. 2.5 gram dextrose per kilogram, hour, *plus* 6 grams Na_2CO_3 in 15 % solution	3	Pancreatico duodenal	0.178	0.095	46 % increase
V. Faintly acid, then alkaline dextrose solution injected	8.8	*a*. No injection		Vena cava	0.063	0.045	
		b. 1.02 gram dextrose per kilogram, hour, *plus* trace of HCl	15	Vena cava	0.013	0.050	10 % increase
		c. No injection	10	Vena cava	0.105	0.065	44 % increase
		d. 1.05 gram dextrose per kilogram, hour, *plus* about 5 grams Na_2CO_3 in 10 % solution	13	Vena cava	0.125	0.125	177 % increase

* Air emboli in injection fluid.

† Blood pressure falling rapidly.

NO. AND GENERAL NATURE OF EXPERIMENT	WEIGHT OF DOG	SUBSTANCE BEING INJECTED INTO MESENTERIC VEIN DURING PERIOD	DURATION OF PERIOD	VESSEL FROM WHICH BLOOD COLLECTED FOR ESTIMATION	DEXTROSE IN BLOOD (AVERAGE OF SEVERAL SAMPLES COLLECTED DURING PERIOD)	LACTIC ACID (IN SAMPLES COLLECTED DURING PERIOD)	PERCENTILE CHANGE FROM NORMAL
	kg.		*min.*				
VI. Alkali alone injected	8.7	*a.* No injection		Pancreatico duodenal	0.182	0.020	
					0.174	0.033	
		b. Injection of 0.6 gram per minute Na_2CO_3 in 15 % solution	12	Pancreatico duodenal	0.186	0.063	110 % increase
		c. Injection of 0.6 gram per minute Na_2CO_3 in 15 % solution	15			0.076	153 % increase
VII. Alkali alone injected	7.2	*a.* No injection		Pancreatico duodenal	0.091		
			5	Pancreatico duodenal	0.084	0.033	
		b. Injection of 0.66 gram per minute Na_2CO_3 in 15 % solution	6	Pancreatico duodenal	0.093	0.093	181 % increase
		c. Continuation of same injection	11	Pancreatico duodenal	0.054	0.088	166 % increase

FURTHER STUDIES OF NERVE CONDUCTION IN CASSIOPEA

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Studies of recent years have shown the importance of hydrogen ion concentration in determining the rate of nerve conduction in the medusa *Cassiopea*. Ordinary distilled water often remains acid even though air, freed from CO_2 by means of soda-lime, has been bubbled through it for seventy-two hours. Accordingly, Prof. George A. Hulett kindly arranged to have 144 liters of distilled water prepared in accordance with his well known method (see Hulett (1)), in his laboratory at Princeton University. This water was sealed in 144 pyrex glass flasks and thus transported to Tortugas. The hydrogen ion concentration of each flask was testedseparately, the range being 0.8 to 1 $\times 10^{-6}$, and the average being 0.9 $\times 10^{-6}$, or 6.04 PH.

Fifty litres of this water were placed in a green glass carboy which had previously held Merck's distilled water; and air freed from CO_2 was bubbled through it at an active rate for seventy-eight hours, after which the water in the carboy had a hydrogen ion concentration of 10^{-8} which it maintained for eight days while 139 experiments were made with it. The alkalinity then declined to a hydrogen ion concentration of 3.16 $\times 10^{-8}$ while 26 other experiments were made; the average hydrogen ion concentration for the series being 1.17 $\times 10^{-8}$, or 7.93 PH. Its alkalinity was probably due to soda derived from the glass carboy, the balance being maintained by a tendency of the water itself to become acid through leakage of CO_2 from the air. Prof. J. F. McClendon found the PH of the Tortugas sea water to range from 8.1 to 8.22 (0.794 to 0.603 $\times 10^{-8}$ CH); and dilution with alkaline distilled water of hydrogen ion concentration 1.17 $\times 10^{-8}$ maintained the normal alkalinity of the sea water even when diluted with its own volume of such distilled water.

The electrical conductivity of the sea water was determined by Kohlrausch's method, using a telephone to detect the minimum sound from an alternating current produced by a small induction coil.

It appears, that when alkaline distilled water of about 8 PH is used to dilute the sea water the decline in rate of nerve conduction is practically the same as the decline in the electrical conductivity of the sea water when similarly diluted. We should remember, however, that the concentration of the sodium, calcium and potassium cations declines in practically the same ratio and we believe we can show that the rate of nerve conduction is proportional to the concentration of these cations rather than to the electrical conductivity of the diluted sea water as a whole.

TABLE 1

Illustrated by figure 1

Rates of nerve conduction when Tortugas sea water is diluted with aerated, alkaline, distilled water having a hydrogen ion concentration of 1.17×10^{-8}

COMPOSITION OF THE SOLUTION	RATE OF NERVE CONDUCTION	ELECTRICAL CONDUCTIVITY OF TORTUGAS SEA WATER DILUTED WITH DISTILLED WATER OF HYDROGEN ION CONCENTRATION 1.58×10^{-8} AT 30°C.
Natural sea water	100.00	100.00
95 volumes sea water + 5 volumes distilled water	96.23	
90 volumes sea water + 10 volumes distilled water	91.44	92.16
80 volumes sea water + 20 volumes distilled water	79.51	81.38
70 volumes sea water + 30 volumes distilled water	73.91	71.53
60 volumes sea water + 40 volumes distilled water	65.72	64.26
50 volumes sea water + 50 volumes distilled water	54.16	54.08

However, Lillie (2) is right in stating that the rate of nerve conduction in *Cassiopea* in diluted sea water does not decline in accord with Freundlich's law of adsorption as I had erroneously supposed. The distilled water used in my previous experiments was slightly acid and this appears to be stimulating in slight dilution, becoming depressant, however, as the dilution is continued, thus giving the semblance of an adsorption curve. Nor did I realize the effects of slight changes in temperature in the various solutions, for the effect of 0.1°C. is readily appreciable.

Lillie advocates an extension of the theory of Faraday and de la Rive, that the transmission of the excitation state from the immediate site of activity to the adjoining resting area is dependent on an electrical local action of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces, such as iron in contact with an electrolyte solution; and if this be true, the rate of nerve conduction should be a function of the electrical conductivity of the medium, and also of the surrounding fluid.

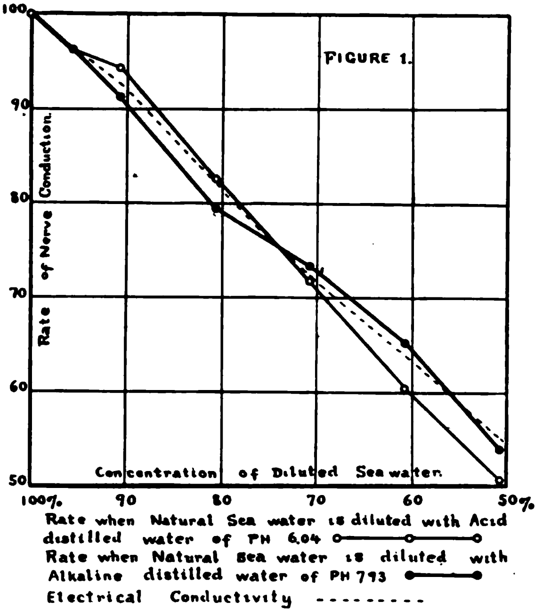
FIGURE 1.

To further test this hypothesis, I determined the electrical conductivity of Tortugas sea water when heated or cooled, and compared it with the rate of nerve conduction at corresponding temperatures.

This shows that the rate of nerve conduction has a temperature coefficient about two and a half times as high as that of the electrical conductivity of sea water; as will appear in table 2.

It will be recalled that Harvey (3) was the first to show that the rate of nerve conduction in *Cassiopea* augments in nearly a straight line as the sea water is heated until about 36° to 38°C. where it suddenly falls off, thus giving a curve resembling that of an enzyme reaction. This has been confirmed by the later work of Mayer (4), and Cary;

and I find that when the rate has begun to fall off, the original rate is not recovered upon cooling. This may possibly mean that the hypothetical enzyme has been partially destroyed; for if this decline were due merely to Winterstein's heat asphyxiation (5) the rate should recover to a greater degree when the medusa is replaced in sea water of normal temperature. Moreover, the high temperature coefficient of the rate

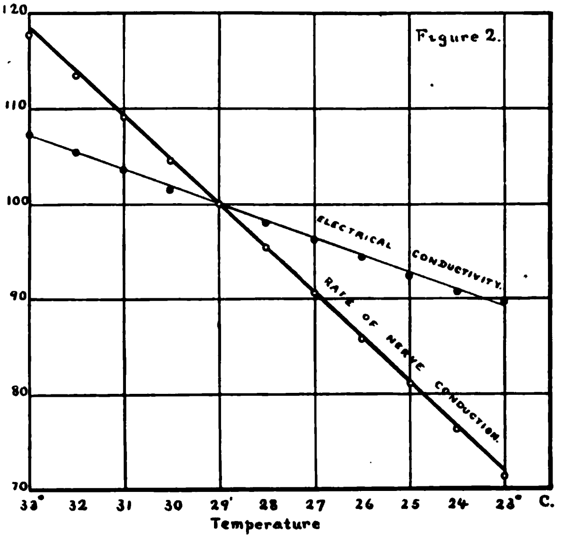

TABLE 2

Illustrated by figure 2

TEMPERATURE C° OF THE SEA WATER	RATE OF NERVE CONDUCTION IN CASSIOPEA. MEAN OF HARVEY, MAYER AND CARY'S OBSERVATIONS	RELATIVE ELECTRICAL CONDUCTIVITY OF TORTUGAS SEA WATER DETERMINED BY KOHLRAUSCH'S METHOD
23	71.30	88.9
24	76.30	90.7
25	81.17	92.6
26	85.80	94.4
27	90.74	96.2
28	95.47	98.0
29	100.00	100.0
30	104.47	101.6
31	109.20	103.5
32	113.40	105.3
33	117.80	107.1

of nerve conduction suggests that we may be dealing with a chemical reaction in which a compound is formed composed of sodium, calcium and some proteid element, (Osterhout (6)), the degree of ionization of which is considerably affected by temperature in the manner suggested by Hardy (7), Quincke (8) and Bayliss (9).

Possibly, also, the negative electrical potential associated with the wave of nerve conduction may increase the surface tension of the alkaline colloidal particles thus reducing their size, rendering them more soluble and thereby increasing the concentration of the reacting ions. (See Mayer, Schaeffer and Terroine (10); and also Hulett (11)).

Contrary to Lillie's hypothesis, we have direct evidence that the rate of nerve conduction may be independent of the electrical conductivity of the surrounding sea water, for I have found (12) that if sea water be diluted with 0.415 molecular $MgCl_2$ the rate of nerve conduction is only slightly more depressed than if the sea water is diluted with distilled water, yet the $MgCl_2$ maintains a nearly normal electrical conductivity, while with distilled water it declines in nearly the same ratio as the dilution. Nor do the experiments I have made with solutions containing some, but not all the cations of sea water, support Lillie's view. For example, if the rate of nerve conduction in 0.647 molecular NaCl is 55, it becomes 100 in 85.3 cc. of 0.6 molecular NaCl + 14.69 cc. of 0.39 molecular $MgCl_2$. Here the electrical conductivity of the solution is somewhat reduced while the rate of nerve conduction is much augmented. This is, of course, a striking instance of Loeb's law of the antagonism between a univalent and a bivalent cation; even though the bivalent cation in this case is magnesium, well known to be a depressant especially for muscular activity in *Cassiopea.*

In slight dilution such as 90 per cent sea water + 10 per cent of distilled water, acid distilled water of hydrogen ion concentration 10^{-6} at 30°C. is slightly stimulating when compared with the effect of alkaline distilled water of 10^{-8} hydrogen ion concentration. In dilution of about 30 per cent distilled water + 70 per cent sea water, and greater, the effect of the acid sea water is reversed for it becomes more and more depressant. This fact will appear upon inspection of table 3.

McClendon (13) found that even when sea water of 8.1 PH is mixed with an equal volume of distilled water of 6 PH the resulting mixture has a PH of 8.09. Thus it is improbable that the stimulating effect of slight amounts of this acid distilled water is due to a change in the concentration of hydrogen ions, especially as the effects of any decided concentration of acid are well known to be depressant.

It seems more probable that the phenomenon is comparable with that observed by Osterhout (14) in plants wherein a weak concentration of HCl is at first stimulating but later after the plasma membrane has been penetrated by the acid it becomes depressant. A slight accession of H ions not sufficient to appreciably change the alkalinity of the sea water appears to be stimulating; and it is possible that it may be unable to penetrate the cell and produces its effect by simple contact with the surface membrane. In stronger concentrations it certainly penetrates the cell and then becomes a depressant for physiological activity.

There are remarkable parallels between the physiological reaction of animals and plants indicating the fundamental kinship of the phenomena. Thus Osterhout (6) has ingeniously calculated the amount of the so-called sodium-calcium-ion proteid formed in *Laminaria* when placed in solutions containing NaCl and $CaCl_2$ mixed in various proportions. He shows that a toxic monivalent cation, such as sodium, causes a fall of resistance, but if a bivalent cation such as calcium be also added the fall of resistance is inhibited. Thus the amount of antagonism may be quantitatively determined.

TABLE 3

Illustrated by figure 1

COMPOSITION OF THE SOLUTION	RATE OF NERVE CONDUCTION WHEN SEA WATER IS DILUTED WITH ACID DISTILLED WATER OF 0.9×10^{-6} H ION CONCENTRATION AT 30°C.	RATE OF NERVE CONDUCTION WHEN SEA WATER IS DILUTED WITH ALKALINE DISTILLED WATER OF 1.17×10^{-9} H ION CONCENTRATION AT 30°C.
Natural sea water of 8.1 to 8.2 PH	100	100
95 cc. sea water + 5 cc. distilled water	96.43	96.23
90 cc. sea water + 10 cc. distilled water	94.4	91.44
80 cc. sea water + 20 cc. distilled water	82.7	79.51
70 cc. sea water + 30 cc. distilled water	71.95	73.91
60 cc. sea water + 40 cc. distilled water	60.41	65.72
50 cc. sea water + 50 cc. distilled water	50.83	54.16

Lillie's hypothesis of electrolytic local action appears to me to be too simple to account for the facts of nerve conduction. As Professor McClendon says there is no such optimum temperature for electrical conductivity, as we observe at about 36°C. for nerve conduction. Nerve reactions must indeed by very much more complex than the "local action" theory would imply, for Cary (15) finds that even if a single one of the 16 or more marginal sense-organs of *Cassiopea* be intact the initial stages in regeneration are decidedly accelerated in comparison with the rate observed when *all* of the sense-organs are removed.

SUMMARY

Nerve conduction is due to a chemical reaction involving the cations of sodium, calcium and potassium; magnesium being non essential to the reaction.

The sodium and calcium cations probably combine together with some proteid element to form a sodium-calcium-ion proteid. The temperature coefficient for the rate of nerve conduction is two and a half times as great as that for the electrical conductivity of the sea water surrounding the nerve, and this may be due to a high temperature coefficient of ionization of the sodium-calcium-ion proteid, which is concerned in the chemical reaction resulting in nerve conduction.

The rate of nerve conduction may be independent of the electrical conductivity of the solution surrounding the nerve, for the rate declines in practically the same manner whether we dilute the sea water with 0.4 m. $MgCl_2$ or with distilled water. In other words whether we maintain the electrical conductivity or reduce it in a ratio nearly commensurate with the dilution.

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THE NATURE AND TIME RELATIONS OF THE FUNDAMENTAL HEART SOUNDS

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I. ARGUMENT

During each cardiac cycle, at least two series of vibrations, of such a frequency that they can be distinguished by the human ear, are initiated within the heart wall, the valves and blood current. They may be designated as the *fundamental heart sounds*. Through the air and solid media of the intervening lung these vibrations are communicated to the thoracic wall. The resultant thoracic vibrations, and not the fundamental cardiac vibrations, are heard in auscultation and recorded by instrumental means. They may be differentiated by the term *thoracic heart sounds*.

In studying the causes, characteristics and chronotropic relations of the sounds by recording mechanisms it is important to have accurate records of the fundamental vibrations. It is questionable, however, whether the vibrations recorded from the thoracic wall are duplicates of the fundamental vibrations originating within the heart. There are theoretical as well as experimental reasons for believing that vibrations may be deleted or added to the fundamental vibrations before they reach the receiver placed upon the chest. As the amplitude of sound vibrations decreases directly with the distance that they are propagated, it is conceivable that vibrations of small amplitude may fail to reach the thoracic wall or become too small to be recorded. The extent to which depression occurs in the apex region depends on the volume of intervening lung, which varies not only in different subjects but, in the same individual, with the phases of respiration. In this way Fahr (1) and Battaerd (2) have explained the frequent variations in contour and grouping of the first sound vibrations taken from the apex region. Owing to the greater distance between the base of the ventricles and chest wall, even larger fundamental vibrations originating in the aorta and pulmonary artery may fail to reach the thoracic wall. Consequently, sound vibrations are not only more difficult to record from the second interspaces but are subject to even greater variation in grouping and contour (2).

Vibrations may be added to the fundamental vibrations in several different ways. In the first place, adventitious respiratory sounds may be incorporated with the fundamental vibrations. This may occur even during expiratory apnea for each change in the volume and position of the ventricles exerts a cardio-pneumatic effect on the lungs capable of creating sound vibrations within lung tissue. In the second place, the thoracic wall may be thrown into vibration by mechanical impacts, as is well known to be the case in thoracic percussion. Conditions for inaugurating such an inherent vibration of the chest wall are especially favorable at the onset of ventricular systole (i.e., during the first sound) owing to the fact that at this time the ventricular apex gives an impact to the thorax. How important this factor is can not be established until the vibration frequency and decrement of the inherent thoracic vibrations are known. That it varies greatly with the type of thorax and vigor of the apex impulse must be logically conceded.

Finally, the relative amplitude of the transmitted fundamental vibrations may be altered. The heart sounds are composed of vibrations varying greatly in their individual periods. The air-containing thorax, which acts as a resonator, may therefore intensify the amplitude of those vibrations with which it is capable of vibrating in unison and reproduce them out of proportion to other vibrations which differ essentially from the inherent thoracic period. In these ways the configuration of the sound groups may be altered or certain waves entirely repressed.

II. METHOD OF RECORDING FUNDAMENTAL HEART SOUNDS

In order to study the fundamental heart sounds as they occur in the heart, we devised a sound transmitter capable of picking up the sound vibrations from any point of the exposed heart and transmitting them to a sensitive recording membrane.

The idea of our sound transmitter was suggested through the previous use of a miniature myocardiograph described elsewhere by one of us (3). Records taken with this apparatus from the contracting auricle often gave an indication of sound vibrations superimposed on the myogram curve. When such a myocardiograph was connected to a very sensitive recording capsule and a side tube in the system was left open, sound vibrations were recorded from any portion of the heart. In order to eliminate to a considerable extent the myogram element, the use of the fixed arm was discontinued, the apparatus being attached to the heart by the movable arm alone. In order to increase the inherent frequency, the segment capsule to which the movable limb was attached was covered by tensely drawn, heavy rubber dam. Thus transformed, the apparatus shows the essential parts diagrammatically illustrated in figure 1 A. The arm, *a*, is fastened by a stitch to any portion of the heart; e.g., ventricle, auricle or aorta. Several of these instruments may be applied simultaneously to the heart in this way, the only limitation in number being that contact of the different transmitters with each other or with other apparatus must be avoided.

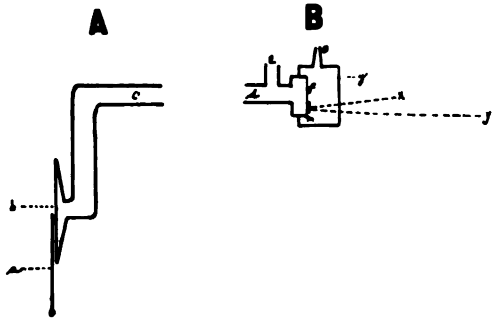

Fig. 1. Diagram showing essential parts of sound recording apparatus. A, sound transmitter; *a*, arm stitched to heart and communicating with diaphragm, *b;* B, protected recording capsule; *d*, receiving tube with side opening, *e*, to eliminate mechanical variations in the system; *f*, rubber film, supporting mirror, *m*. Capsule is inclosed by housing with front plane window, *g*, and a vent, *o*, for equalizing pressures on membrane.

The sound transmitter is connected by soft rubber tubing about 30 cm. long[1] with a Frank capsule which is 10 mm. in diameter and covered with a delicate rubber film. This film is made by passing the opening of the capsule through a strand of rubber cement and subsequently stretching the partially dried film in order to render it tense. Before the film has completely dried, a tiny mirror (1 to 2 mm. square) is placed upon it and allowed to adhere. Such a membrane exposed to the air is responsive not only to sound vibrations communicated by the tube, *d*, but is so sensitive to accidental sound waves which impinge upon the side exposed to the room that, at certain times, it is almost impossible to obtain clear heart sound records. To obviate this objection, our earlier experiments were carried on only at night when accidental noises were reduced to a minimum. Recently, it has been found possible, however, to modify the apparatus in such a way that it does not respond to accidental room vibrations of considerable intensity. This modification consists in inclosing the membrane by a housing, *g*, having a front window of perfectly plain glass. In this housing is a conical vent, *o*, tapering exteriorly to an aperture only 1 mm. in diameter. This is sufficient to equalize the slight pressure variations within the housing but prevents the entrance of extraneous sound vibrations.

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[1] This short tube introduced a delay of only 0.00099 seconds in sound transmission.

III. THE EFFICIENCY AND LIMITATIONS OF THE SOUND RECORDING MECHANISM

It is of primary importance to determine whether the sound recording system utilized is capable of responding faithfully to all the sound vibrations that may originate within the heart and, above all, to satisfy ourselves that the errors attributed to the interposition of the thoracic wall are not reduplicated by the recording system. In relation to this question the vibration frequency is of paramount interest. The complete system had a vibration frequency ranging from 150 to 250 per second, depending on the tension of the rubber stretched over the transmitter, as well as on the tension given the rubber film of the recording capsule. Care was taken that the frequency did not fall below the former figure but it was not technically possible at the time these experiments were made to increase the frequency above 250 per second. While such a system is capable of giving *an indication* of vibrations occurring at any frequency not exceeding its inherent rate, it is not capable of vibrating with an amplitude devoid of resonance effects when the frequency exceeds about 100 per second. This was established by its ability to record tuning-fork vibrations of different amplitude and period.

A consideration of the average frequency of the sound waves recorded from man up to the time that this research was inaugurated, led to the conclusion that the sounds of the heart are composed of vibrations having a frequency less than 100 per second. Thus, Gerhartz (4) calculated that the average frequency of the first sound in Einthoven's curves was 39.4 and of the second sound, 47.5 per second, while Einthoven (5) himself gave 50 per second as the frequency of the third sound. Frank (6) and Lewis (7) found that the average frequency of the two sounds approximated 40 per second although the latter observer sometimes found frequencies as high as 70 per second for the first, and 86 per second for the second sound. Consequently, at the time these experiments were undertaken, we were under the impression that these capsules were capable of recording without error all the individual vibrations occurring within the heart.

Recently, Battaerd (2) has found that some of the sound vibrations taken from man by a galvanometer string capable of vibrating 3000 times per second have a period as short as 0.001 second. It is quite evident that our capsules are entirely incapable of responding to vibrations even approximating such a figure. It may be emphasized, therefore, that our description of the vibrations occurring within the heart is limited to the predominant vibrations of longer period. Whether additional vibrations of shorter period actually exist within the heart or whether the short vibrations found in the thoracic sounds by Battaerd are due to overtones or harmonics originating in the thoracic cavity, must be subsequently determined by other methods.

After considerable experimentation we came to the conclusion that it is unwise to adopt special means of increasing the damping of the capsules. Consequently, they are not quite aperiodic. This introduces the possibility that any sudden, mechanical impact derived from the heart may set the system into vibration at its own inherent rate. Experimental tests, however, indicate that this does not occur when an adequate side opening is maintained in the system. A sound transmitter was attached by a thread to a 100 per second tuning-fork which was so mounted that its movements were simultaneously recorded with the corresponding vibrations of the optical capsule. If the vibrating tuning-fork was then rapidly moved through a distance much greater than is possible when attached to the heart no inherent vibrations were added to the sound record and the periods of vibrations remained unaltered, the only change being a modification of the relative amplitude of the recorded sounds (fig. 2). This may be explained by the fact that the decremental quotient[2] of the inherent vibrations was favorable. Thus, in a typical experiment the quotients 1.54, 1.33, 1.33, 1.37, 1.23, 1.41 and 1.5 (average 1.38) were found in comparisons of successive vibration phases. The average quotients found in the capsules used at different times ranged from 1.2 to 1.48.

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[2] By the *decremental quotient* is understood the quotient obtained by dividing the amplitude of any vibration phase by the amplitude of the next vibration phase.

In addition to these instrumental checks upon the efficiency of the apparatus, many precautions were taken during the experiments in order to prevent the communication of accidental vibrations to the transmitter or connecting tubing. The sound transmitter attached to the heart was suspended by its horizontal arm through a ring of very thin rubber, which enabled the entire apparatus to follow the movements of the heart readily and yet give the swing of the entire instrument a very long period. The connecting tube was similarly suspended so that its sway also had a low frequency. To annul vibrations of pulmonary origin the records were taken during the apnea following a temporary lull in artificial respiration during which phase the lungs were completely collapsed. To avoid friction rubs of the moving heart, this organ was allowed to remain intact within the pericardium, a small window only being cut over the point to which the arm of the sound transmitter was attached. In the earlier experiments the entire animal and operating boards were also suspended by appropriate springs in order to avoid the transmission of vibrations by way of the building. This precaution was later found to be unnecessary since these vibrations apparently are damped by the resiliency of the animal's own body.

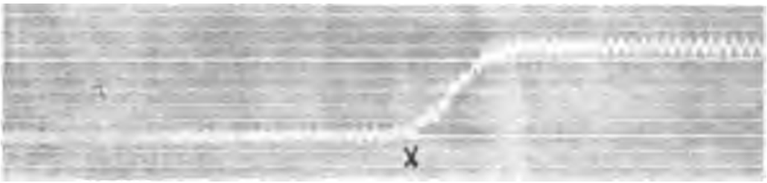


Fig. 2. Curve showing the effect of a sudden movement of a vibrating tuning fork at x (lower curve) on the vibrations recorded by an optical capsule (upper curve).

Finally, the efficiency of the system was checked by auscultation just before records were taken. If a stethoscope is connected with the sound transmitter each sound has a quality not unlike that heard over the thoracic wall. Records were taken only when the sounds were clear and free from friction rubs or other accessory noises. In one instance the records obtained by the optical capsules were also compared with those obtained by substituting a microphone circuit connected with a highly damped galvanometer string capable of vibrating approximately 1000 times per second. The similarity of the curves gave final evidence of the adequacy of the recording mechanism for studying the fundamental heart sounds.

IV. THE COMPONENTS OF THE FUNDAMENTAL HEART SOUNDS AND THEIR TIME RELATIONS

The sound vibrations responsible for the fundamental heart sounds heard on auscultation were recorded from different areas of the heart and large vessels in twenty-two dogs. In each case both the dynamic condition of the heart and the total peripheral resistance were varied experimentally in order to preclude the possibility that the recorded vibrations might not correspond to those found in the intact heart. Records were also obtained with recording capsules varying in their sensitivity and by communicating systems with different degrees of lateral opening. When sound vibrations from different cardiac regions were simultaneously recorded the capsules were repeatedly interchanged. These precautions were taken in order to discount possible unrecognized errors in the apparatus and technic. In this manner a large number of tracings were obtained in which the fundamental sound vibrations were compared with the dynamic and electrical changes in the heart. It is our purpose in this paper to analyze only what may be regarded as the normal ventricular and aortic sounds from the two sides of the heart.

1. The analysis of sound records. The vibrations assembled by the sound transmitter attached to the heart are a mixture of mechanical impacts and sound vibrations, but by appropriately adjusting the lateral opening to the sensitiveness of the recording capsule, a differential record of sound vibrations may be obtained. The accomplishment of this end involves the same principles and difficulties that are discussed by us (8) in the case of recording sounds from the thoracic wall. It is not always possible, therefore, to obtain records entirely free from mechanical oscillations while, in addition, extraneous, accidental vibrations of small amplitude may recur throughout the curve in spite of the best precautions. This may be illustrated by reference to the illustrations accompanying this article. In the middle curve of figure 4, almost no extraneous vibrations or mechanical variations are introduced, the curve presenting a pure heart sound record. In the upper record mechanical variations are also absent, as indicated by the straight line between sounds. There are, however, a number of small accidental vibrations labelled x. In the records shown in figure 5, A and B, the curves are complicated by the additional presence of grosser mechanical variations which owe their existence to the fact that the lateral opening in the recording system was not quite adequate. Until it becomes technically possible to obviate these distortions in all cases the eye must be trained to distinguish the essential from the accidental and mechanical vibrations, much as the ear is trained in auscultation to disregard sounds for which it is not listening.

2. The components of the first ventricular sound. The vibrations recorded from different regions of the right and left ventricle show no essential differences (cf. figs. 5 A and 5 B); hence, they may be referred to in common as the *ventricular sounds.*

The first ventricular sound is composed of a series of five to thirteen irregular vibrations the periods of which range from 0.004 to 0.054 second. As their maximum period rarely exceeds 0.024 second, it is doubtful whether vibrations of longer period really may be classed as entering into the sound. The duration of the sound varies from 0.05 to 0.152 second (cf. illustrations and table 1). The components of the first sound are clearly discernible upon careful inspection of the various illustrations in spite of the fact that they were somewhat reduced in size during their reproduction. The sounds are shown in enlarged form in the upper record of figure 3. The sound starts with an *introductory vibration,* A. Occasionally there are two of these vibrations (cf. figs. 5 and 6). This is followed by a group of five or six *main vibrations,* B, the onset of which is always distinctly indicated by a sharp deflection of the light band, either in an upward or downward direction. They are followed by a group of *final vibrations,* C, varying in number.

Both the number and individual amplitudes of the vibrations in each group depend on the vigor of ventricular systole but their period is unaffected, as shown in the two segments of figure 4. Segment A shows the ventricular sounds recorded shortly after exposing the heart. In such cases the effective venous pressure and the arterial pressure are reduced; consequently, the heart is somewhat hypodynamic. Nevertheless, the sounds on auscultation are clear and dis-

TABLE 1

Ventricular sounds

EXPERIMENT	PLACE TAKEN	FIRST SOUND			SECOND SOUND			RATIO FIRST: SECOND SOUND DURATION
		Number of vibrations	Range in period	Duration	Number of vibrations	Period	Duration	
			sec.	*sec.*		*sec.*	*sec.*	*sec.*
C110	Right ventricle	5	0.0026–0.017	0.092	3	0.0115	0.028	3.2: 1.0
C113	Right ventricle	6	0.004 –0.027	0.152	4	0.009	0.047	3.2: 1.0
C115	Right ventricle	6	0.006 –0.010	0.050	3	0.007	0.015	3.3: 1.0
C118	Right ventricle	5	0.006 –0.018	0.063	2	0.009	0.018	3.5: 1.0
After adrenalin	Right ventricle	12–13	0.009 –0.018	0.126	3	0.009	0.027	4.7: 1.0
After adrenalin	Right ventricle	10–11	0.006 –0.036	0.063	3	0.009	0.036	1.7: 1.0
C120. After adrenalin	Right ventricle	12–13	0.006 –0.036	0.099	4	0.009	0.018	5.5: 1.0
C122	Left ventricle	8–9	0.008 –0.024					
C123	Right ventricle	7–8	0.0062–0.0187	0.10	4	0.0125	0.056	1.7: 1.0
C124	Left ventricle	7– 9	0.012 –0.018					
After adrenalin	Left ventricle	8–13	0.009 –0.024					
C125	Right ventricle	7– 9	0.007 –0.021	0.080	5–6	0.008–0.024(?)	0.044	1.8: 1.0
C127	Left ventricle	7– 8	0.009 –0.054 (?)	0.068	3	0.013	0.025	2.7: 1.0

TABLE 2

Aortic sounds

EXPERIMENT	PLACE TAKEN	FIRST SOUND			SECOND SOUND			RATIO FIRST; SECOND SOUND DURATION
		Number of vibrations	Range in period	Duration	Number of vibrations	Period	Duration	
			sec.	*sec.*		*sec.*	*sec.*	*sec.*
C111	Pulmonary	5– 6	0.012–0.018	0.058	3	0.020	0.035	1.6: 1.0
C112	Pulmonary	12–19	0.006–0.02	0.065				
C115	Aorta				7	0.007	0.045	
C116	Aorta	9	0.012–0.018	0.108	6	0.012	0.060	1.8: 1.0
C116	Pulmonary	7	0.006–0.018	0.048	10	0.009	0.042	1.1: 1.0
C118	Aorta	12–13	0.008–0.020	0.153	4	0.015	0.072	2.1: 1.0
After adrenalin	Aorta	9–10	0.008–0.018	0.126	4	0.018	0.063	2.0: 1.0
C120	Aorta	4– 5	0.009–0.018	0.028	2	0.009	0.018	1.5: 1.0
C125	Pulmonary	4– 5	0.007–0.013	0.039	2	0.007	0.021	1.8: 1.0
C127	Aorta	7– 8	0.009–0.018	0.081	2	0.018	0.036	2.2: 1.0

tinct and equal in intensity those normally heard over the chest wall. The recorded vibrations are few in number but comprise all the components of the first sound. They start with an *introductory rise*, A, followed by a group of four or five *main oscillations*, B, and a few final vibrations, C, discernible by very careful inspection. Segment B shows a similar series of sounds intensified in amplitude owing to the stimulating action of adrenalin on the heart. The introductory variation, A, is larger, the main oscillations, B, increase both in number and amplitude, and the final variations, C, become distinct. These three components which have been designated as the *introductory*, the *main* and the *final vibrations*, respectively, we consider characteristic of fundamental ventricular sounds. The introductory vibrations may be entirely lacking even during vigorous cardiac action and the final vibrations are wanting in essentially hypodynamic hearts. The main group of vibrations, though reduced in number of individual waves, is the last to persist in dying hearts.

A few words must be incorporated in regard to certain vibrations preceding the main group in phonocardiograms taken from the apex region of man. Fahr (1) described these variations and designated them the "initial variations." Their period is longer and they occur 0.02 to 0.04 second, exceptionally 0.09 to 0.10 second, before the main vibrations. Similar waves are recognized in tracings published by Bull (9), Battaerd (2) and in some of our unpublished tracings from men. We are in doubt whether these variations correspond to our introductory variations or to still another coarse variation sometimes preceding the introductory rise. Owing to this uncertainty we have employed the term "introductory" in our description to avoid possible confusion in the future. Of one fact we are certain—if the "initial vibrations" precede our "introductory variation" they are presystolic in time and have no claim to being a part of the first heart sound.

Fig. 3. Records of ventricular sounds (upper record) and aortic sounds (lower record) enlarged three times from original and showing details of sound vibrations. *A*, introductory vibrations; *B*, main vibrations; *C*, final vibrations in ventricular sound record; C_1, first component; C_2, second component of main vibrations in aortic record; *2S*, second sound.

Fig. 4. Two segments of a record showing aortic sounds (upper curve), right ventricular sounds (middle curve) and their relation to the electrocardiogram, Lead II. A, during hypodynamic heart action consequent to opening chest; B, during action of adrenalin (one-half actual size). *P*, relative alignment of points; *X*, accidental vibrations; S_1, first sound; S_2, second sound; *A*, introductory vibration; *B*, main vibrations; *C*, final vibrations of ventricular first sound; C_1, first component; C_2, second component of first aortic sound. Ink lines show synchronous points on three curves.

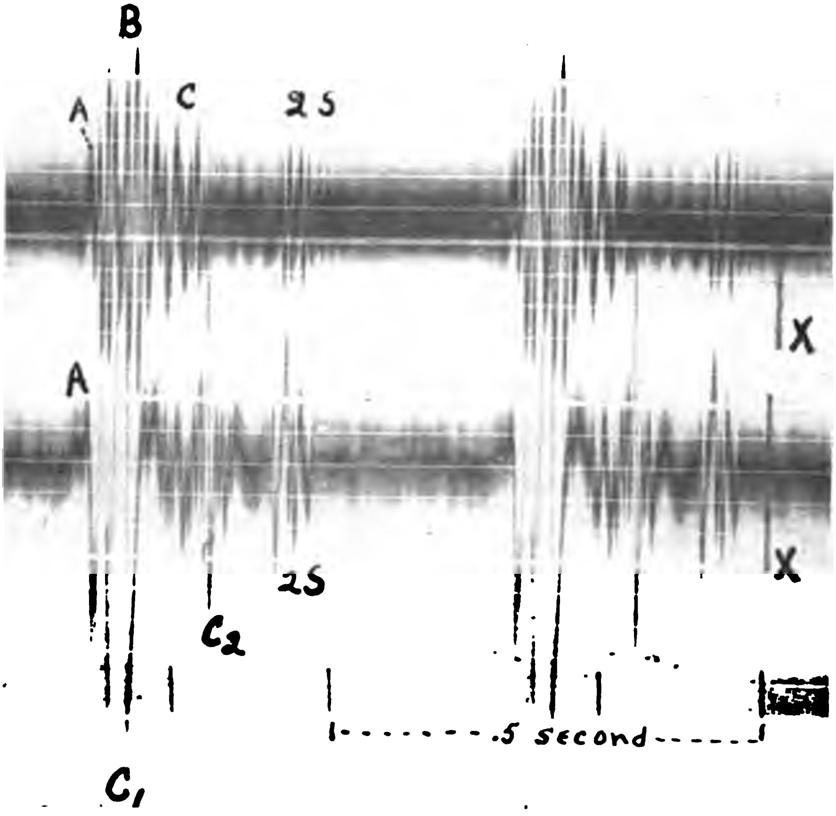
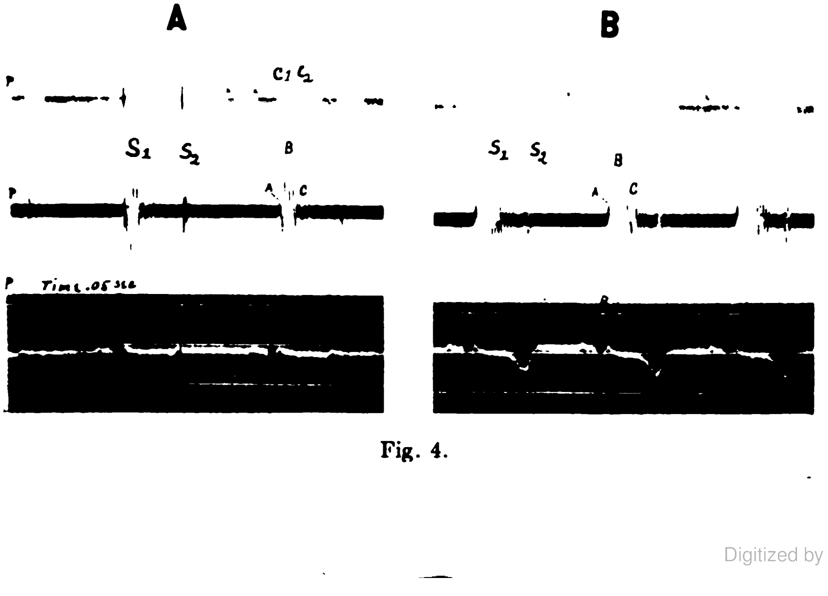

B
A
C
2S
X
A
2S
X
C2
C1
.5 second

Fig. 3.


A
B
P
C1 C2
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A C
A C
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Fig. 4.

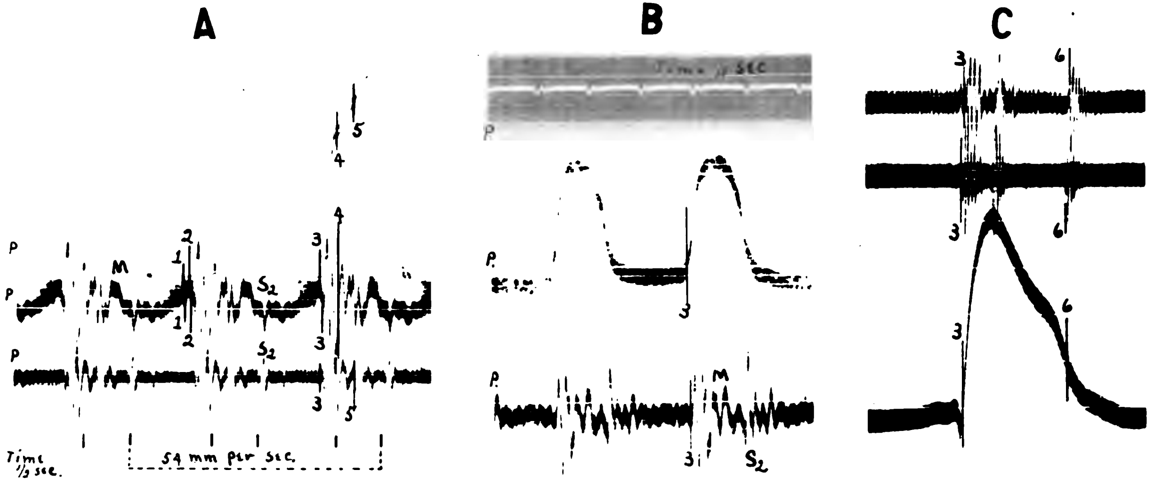

Fig. 5. Three segments of records from three different experiments showing relation of sounds to intraventricular pressure. (Three-fifths actual size.)

A. Left intraventricular pressure (upper curve), right ventricular sounds (middle curve), aortic sounds (lower curve). *P*, relative alignment of points; *M*, mechanical oscillations; S_2, second sound; Lines *1, 2, 3, 4* show corrected time relations of first sounds to intraventricular pressure curve.

B. Left intraventricular pressure and left ventricular sounds from apex of heart; letters same as before.

C. Aortic sounds (upper curve), pulmonary arterial sounds (middle curve), right intraventricular pressure (lower curve), showing two components of first sound in both aortic and pulmonary arterial curves; numbers same as before.

3. The relations of the components of the first ventricular sound to dynamic events. As the nature and number of introductory vibrations varies in different records of the first sound, it is of prime importance to determine what point may be taken as the beginning of the first sound. This question is of more than academic interest, involving, as it does, the relation of sounds to other events, and the point that may be taken as a criterion of the beginning of ventricular systole. These questions may be analyzed by a careful comparison of the three components of the first sound with the dynamic events of the cardiac cycle.

The relation of the first sound to the intraventricular pressure recorded by an optical manometer described by one of us is shown in the records of figure 5 taken from three different experiments. The relations are indicated by corresponding lines corrected for slight errors in the vertical alignment of apparatus. The *introductory vibration,* begins a trifle (average 0.018 second), but distinctly *before* the rise of intraventricular pressure (lines 1, 2). This is true of the right as well as the left ventricle. The *main vibrations* begin almost synchronously with the rise of intraventricular pressure (lines 3). They reach their greatest amplitude before the ejection period begins (lines 4). The *final vibrations* occur during the early portion of the ejection period. Recorded synchronously with the aortic pressure (fig. 6) it is found by measurement that the *introductory variations* precede the preliminary rise of arterial pressure described by Frank by 0.025 to 0.05 second (lines 1). The *main oscillations* begin with or very shortly after the rise of this preliminary variation (lines 3); i.e., during the isometric period of the ventricle.[3]

The vibrations attain their maximum amplitude (lines 4) just before the aortic pressure begins to rise, showing that they diminish rapidly as soon as ejection occurs. The *final vibrations* appear during the rise of the primary wave of aortic pressure but end shortly after its summit is reached (lines 5).

A comparison of the first sound with the right auricular myogram (fig. 7) shows that the introductory vibration begins a short interval (average 0.018 second) after the termination of auricular systole, as indicated by the apex of the auricular myogram.

Two facts are evident; first, that the introductory vibration or

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[3] The exact relation of the introductory vibrations is often difficult to determine, owing to the small size of the preliminary vibration.

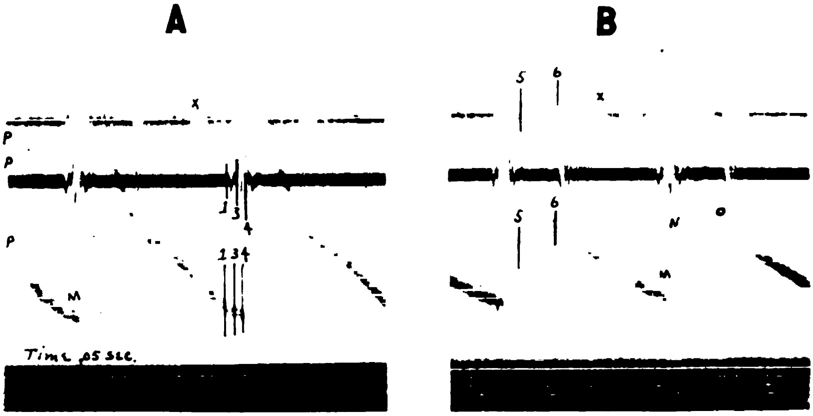

Fig. 6. Two segments of a record showing time relations of different components of the aortic sounds (upper curve) and right ventricular sounds (middle curve) to intra-aortic pressure (lower curve). (One-half actual size.) A, during hypodynamic cardiac action; B, after compression of aorta; other numerals and letters same as before. *M*, preliminary vibration; *N*, primary oscillation; *O*, incisura of aortic curve.

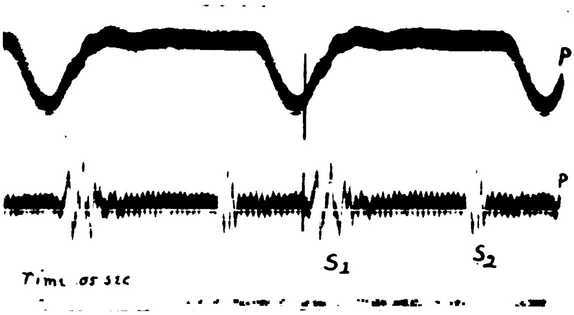

Fig. 7. Segment of record showing relation of first right ventricular sound to end of auricular systole, as indicated by apex of auricular myogram (upper curve); second sound accentuated. (About three-fourths actual size.)

vibrations occur during auricular relaxation and a variable, though short, interval before the rise of intraventricular pressure; and second, that the main vibrations, whether they begin with an upward or downward movement, coincide with the first rise of intraventricular pressure. Either one of two conclusions may be drawn: (1) That the ventricular muscle gives evidence of contraction by an introductory sound vibration before it is capable of elevating the intraventricular pressure; or (2) that the introductory sound vibration does not represent the onset of ventricular systole. We have no evidence enabling us to decide between these alternative conclusions. Inasmuch as the onset of the introductory vibrations is so variable and so frequently absent in records from the thoracic wall, and inasmuch as the onset of the main oscillations is so precisely related to a definite dynamic event; viz., the rise of intraventricular pressure, we propose to utilize the first of these main oscillations in time comparisons.

4. The relation of the first sound vibrations to the electrocardiogram, Lead II. The relation of the main vibrations of the thoracic sounds to the waves of the electrocardiogram have been frequently studied. Kahn (10), utilizing Weiss's phonoscope, found that these vibrations began during the descending limb of R_{II} or 0.01 second before its completion. Bull's results (9) are essentially in agreement. He found that the first sound began 0.03 to 0.04 second after the rise of the R wave; i.e., near its completion. According to Battaerd (2) the first sound follows the rise of R_{II} by 0.02 to 0.065 second, the interval apparently depending on the intensity of the sound and the sensitiveness of the microphone transformer. The results of Lewis (7) and Fahr (1) are in essential agreement but Fahr considers the initial vibration as indicative of the onset of ventricular systole, thereby placing this event on the ascending limb of R_{II} and occasionally, it must be confessed, even before the rise of R_{II} (cf. fig. 3 in his article). Records taken by Williams (personal communication) indicate that the onset of the main oscillations may begin near the R_{II} peak; i.e., 0.025 second after the onset of R_{II}.

A study of our records of the fundamental sounds (cf. fig. 4) shows that the introductory variations begin 0.01 to 0.02 second after the rise of R_{II}; i.e., on the ascending limb. The main oscillations, however, never begin before the peak of R_{II} has been reached. They occur from 0.03 to 0.045 second after the onset of R_{II}; i.e., on the descending limb or after its completion.

5. The characteristics of the second ventricular sound. The second ventricular sound, when compared to the first, is composed of a much simpler set of vibrations (fig. 3), averaging two to six in number and lasting 0.015 to 0.056 second. The ratio to the duration of the first sound varies from 1 : 1.7 to 1: 5.5 (table 1). The vibrations rapidly reach a maximum and show a rapid decrement. Their number and amplitude depend on the vigor of ventricular systole (fig. 4) as well as on the height of diastolic pressure (fig. 6). Their period is quite constant in each case and varies from 0.007 to 0.0125 second in different animals, as shown in table 1.

The maximal amplitude of the second vibration, as a rule, is less than that of the corresponding first sound (figs. 3 and 4, A) but when arterial pressure is greatly increased, as during the action of adrenalin (fig. 4, B) or on compression of the aorta (fig. 6), it may very nearly equal that of the first sound. Upon auscultation a distinctly accentuated second sound is heard (cf. also fig. 7, in which an accentuation of the second sound was a natural occurrence).

In some cases the vibrations of the second sound are barely recognizable. This occurs in some cases when cardiac action, as indicated by the contour of the intraventricular curve, is apparently normal. This is the case in figure 5 A, when the second sound is hardly indicated in spite of considerable closure of the transmitting system. In such cases they become more distinct again after the administration of adrenalin.

6. The time relations of the second ventricular sound. Figures 5 A, B show clearly the relation of the second sound to the intraventricular pressure curve. The vibrations of the second sound begin an extremely short interval after the pressure begins to fall in the relaxation period. Comparison with the aortic pressure curve (fig. 6) confirm these relations. The first vibration of the second sound corresponds very nearly to the beginning of the incisura (lines 6). The vibrations are completed 0.015 to 0.025 second after the bottom of the incisura is reached. Comparison with intra-auricular pressure curves showing a fall of pressure early in ventricular diastole (fig. 8) indicates that the second sound precedes this fall by 0.06 to 0.08 second (lines 6, 7).

The relation of the second ventricular sound to the T wave was found to be extremely variable. Occasionally the onset corresponded with remarkable precision to the end of the T wave (figs. 4 A, 9). At other times it began before this wave had reached its end. When the T wave is negative, as after the administration of adrenalin, the second sound may begin before the trough of the T wave is reached (fig. 4 B). This, it may be added, occurred in spite of a shortening of the R–T interval.

7. *The components of the first aortic sound and their time relations.* The first sound recorded directly from the aorta or pulmonary artery as a rule, contains more vibrations than the corresponding first ventricular sound. Its duration varies from 0.028 to 0.15 second (table 2). In some records the configuration of the first aortic sounds does not differ essentially from that of the first ventricular sounds but in most records essential differences exist which persist upon exchange of registering capsules (fig. 3). The aortic vibrations, like those from the ventricle, may or may not start with an *introductory variation*, A. It may be present in one record and not in the other when synchronously recorded. Hence, if we attempt time comparsions between the two sounds from the first recorded introductory variation discrepancies occur. Following the introductory variation we recognize a group of large oscillations that will be referred to as the *first component*, labeled C_1 in figures 3 and 4. These oscillations start precisely with the main vibrations of the first ventricular sound. The time relations established for the latter, therefore, also hold for the first aortic sound. The periods of this component do not correspond vibration for vibration with the ventricular sound (figs. 3, 4, 5 A, 6), nor is the maximum amplitude reached synchronously. As a rule, the frequency is greater and the maximum reached earlier in the aortic curves (fig. 3). Following these vibrations comes a *second component* (marked C_2 in figs. 3 and 4) which generally reach a second maximum amplitude during the ejection period. This is shown by their comparison with the intraventricular and aortic pressure curves (5 A and 6, lines 5). In some experiments the vibrations of the second component are continuous with those of the first component (fig. 4 B) but, owing to the fact that two distinct sets of large vibrations occur during the sound, they give it a configuration essentially different from the first ventricular sound. A peculiar variation of this component is found in some experiments; viz., that it may be apparently dissociated from the first component and occur as an isolated set of vibrations during midsystole (figs. 3, 5 A); in other words, the first sound is reduplicated. Occasionally it is displaced so late into systole and so near the second sound that the first impression is one of a reduplicated second sound, a fact rendered improbable by its time relations to dynamic events. Neither the cause nor the significance of this dissociation is clear.

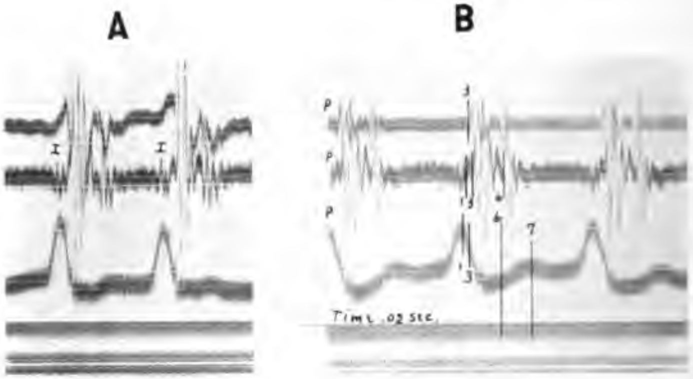

Fig. 8. Two segments of a record showing the time relation of the pulmonary sounds (upper curve) and aortic sounds (middle curve) to the right intra-auricular pressure (one-half acual size). Letters and numerals same as before; 7, diastolic fall of intra-auricular pressure following opening of tricuspid valve. First segment normal; second segment during depressed cardiac action due to pituitrin.

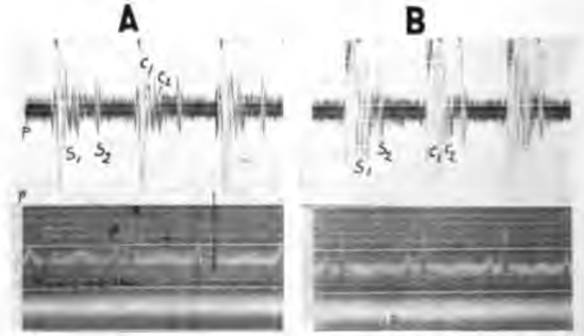

Fig. 9. Two segments of record showing effect of adrenalin (second segment) on components of first and second pulmonary sounds and time relations to electrocardiogram, Lead I. Lettering same as before. (Three-fifths actual size.)

The possibility must, of course, be borne in mind that in such cases the dissociation may be virtual and not real, that between these components small vibrations exist which are too feeble to record. The second component is frequently absent in records from the pulmonary artery but, again, may be as prominent as that of the aortic record (fig. 3 C). Of the influences which modify the vibrations of the first aortic sound a number may be noted. Following the administration of adrenalin, during asphyxia and after aortic compression the amplitudes of both components increase (figs. 4, 6, 9). The second component is entirely absent when arterial pressure is low (fig. 8, B). In such cases it may frequently become prominent upon compression of the aorta (fig. 6). In hypodynamic hearts the first component is smaller in amplitude than the second, giving the second component an accentuated appearance. Compression of the aorta and administration of adrenalin in such cases intensify the first component and reestablish the usual relations in amplitude.

8. The characteristics and time relations of the second aortic sound. The second aortic sound consists of a series of two to ten large decrescendo vibrations (fig. 3). Its duration varies from 0.018 to 0.072 second and is, therefore, considerably shorter than the first aortic sound, the ratios of the two sounds varying from 1: 1 to 2: 1 (table 2). Its onset coincides with the second ventricular sound, indicating the beginning of diastole, as shown by comparison with the intraventricular and arterial pressure curves (figs. 5 A, 6). The relation to the electrocardiogram is shown in figure 9.

The second aortic differs from the second ventricular sound in that the amplitude of its vibrations is larger than those of the second ventricular sound and usually exceeds those of the first aortic sound (figs. 3 and 4). The latter fact accords with auscultatory findings—that the second arterial is louder than the second ventricular sound. This relation is not always found, however. The first aortic sound may consist of vibrations greater in amplitude than those of the second sound. This is the case in figure 6. This variation can not be attributed solely to variations of blood pressure because the amplitude of the pulmonary sound frequently equals that of the aortic sound (fig. 5 C) and certainly the inequality of pressure in the two systems is evident.

SUMMARY

In order to investigate the nature, causes, variations and time relations of the heart sounds it is of greatest importance to record accurately the fundamental vibrations that arise in the heart and large vessels. For a number of reasons it is questionable whether the heart sounds recorded from the thoracic wall are composed of vibrations identical with those occurring within the heart. We therefore devised a new form of sound transmitter which can be applied to any spot on the heart or large vessels and transmits the fundamental sound vibrations to a modified form of Frank's segment capsule. By simultaneously recording the pressure changes within the auricles, ventricles and aortae by an optical manometer, and the electrical variations of the heart by Williams's model of Einthoven's string galvanometer, it has been possible to relate precisely the fundamental sound vibrations to the dynamic and electrical events occurring during each cardiac cycle.

The first ventricular sound so recorded may be described as consisting of three continuous elements:

1. One or two *introductory vibrations* which begin during auricular relaxation, precede by a variable interval the onset of intraventricular pressure, and occur during the rise of the R_{II} wave of the electrocardiogram.

2. The *main vibrations*, composed of seven to thirteen vibrations of irregular period and amplitude which begin with the onset of the pressure rise within the ventricle, reach their maximum during the isometric period and begin on the descending limb of the R_{II} wave of the electrocardiogram.

3. Three to six *final vibrations* which occur during the period when the ejection of blood from the ventricle takes place and during the R–T interval of the electrocardiogram.

The first aortic and pulmonary sounds are also divisible into three components which do not correspond, however, in period, amplitude or grouping to those of the first ventricular sound and, consequently, give the curve a configuration essentially different from that of the first ventricular sound. The components are:

1. One or two *introductory vibrations* comparable to similar vibrations in the first ventricular sound.

2. The "*first component*" of main vibrations, consisting of a group of vibrations which begin synchronously with the main vibrations of the first ventricular sound and reach their maximum during the isometric period.

3. The "*second component*" of main vibrations, occasionally consecutive to the first component but often dissociated from it, so as to give the sound a reduplicated character. These vibrations occur during

the ejection period of the ventricle and reach a maximum amplitude during a variable portion of this period.

The second sounds from both the arteries and ventricles consist of a short, simple series of vibrations, occurring precisely at the onset of diastole, as evidenced by their occurrence at the beginning of the diastolic fall of intraventricular pressure and the onset of the aortic incisura. They precede the opening of the a–v valves by a considerable interval, as evidenced by their comparisons with the diastolic fall of pressure in the auricle. They may, but do not necessarily follow the T_{II} wave of the electrocardiogram. The second aortic and pulmonary sounds differ from the synchronously recorded second ventricular sounds by their larger amplitude and sometimes by the greater number of vibrations.

It would be possible, and perhaps permissible, to construct a hypothetical explanation as to the causes of these differences in the fundamental ventricular and aortic sounds. It seems preferable, however, to reserve such speculation until further experimental data are available.

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THE DEPRESSOR FUNCTION OF THE THORACIC SYMPATHETIC NERVE AND ITS CONNECTIONS

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THE VASCULARITY OF THE KIDNEYS AND INTESTINES DURING SPLANCHNIC DEPRESSION

Provided that currents of low intensity and frequency are employed, the stimulation of the intact thoracic sympathetic, greater splanchnic or abdominal sympathetic nerve, invariably leads to a very decisive fall in blood pressure. The same result may also be obtained by the excitation of the central end of one or the other of these nerves, and besides, this reaction does not disappear after the opposite thoracic sympathetic, both vagi and both phrenic nerves have been cut (1). My attention has recently been called to a preliminary notice of Auer and Meltzer (2) in which it is stated that the electrical stimulation of the central end of the splanchnic nerve causes an unmistakable drop in blood pressure. I am more than glad to permit my data pertaining to this point to serve in complete confirmation of the results of these authors.

The perseverance of the depressor action of the thoracic sympathetic nerve and its branches after the division of the nerves enumerated previously seems to show that this reaction is participated in by certain vascular areas which are not under the direct control of the splanchnic system. The deduction must therefore be made that the depression is not due to a local splanchnic reflex, but is dependent upon a vaso-dilatation of wide extent. This assumption, however, does not preclude the likelihood that the blood vessels of the splanchnic organs ordinarily take part in the depression, in fact, it has been pointed out by me that they do, because the division of the abdominal nerves diminishes the intensity of the reaction. In the succeeding pages a number of experiments are described which have a direct bearing upon this question.

As is true in the case of the depressor cordis, the circulation of the

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abdominal organs may be affected in two ways, namely, actively and passively. Under ordinary conditions of experimentation, however, it is quite impossible to distinguish between these reactions, because they coincide in time and their general character is practically the same. Realizing this difficulty, the experiments to be described have been conducted in such a way as to enable me first of all to detect the latter type of changes. Their analysis has been attempted oncometrically as well as by the direct measurement of the blood supply of the kidneys and intestines. To begin with, however, they consisted merely in the registration of the volume of the kidneys while stimulation of the central end of one or the other thoracic sympathetic nerve was resorted to. The experiments were performed upon dogs, ether being used for the narcosis. The left femoral artery having been connected with a mercurial manometer, the abdomen was opened and the kidneys isolated sufficiently to be placed in oncometers later on. The diaphragm was then pierced in the region of the crura so that shielded electrodes could be applied to the central end of one of the thoracic sympathetic nerves. The stimulations were made at intervals, a Du Bois-Raymond coil and three-volt battery being made use of.

The possible criticism that the fall in blood pressure might be due to a local splanchnic reflex, I have sought to meet first of all by a complete isolation of the nerve by means of rubber membrane. Moreover, the strength of the current was always so slight that a direct stimulation of neighboring tissues could not have resulted. In many cases I have separated the nerve from its surroundings by dividing the lower thoracic roots so that the electrodes could be applied at least two inches away from any nervous connections. These details are in a way self-understood and I mention them merely to make certain of this point.

That the reaction is not confined to local vascular areas is also indicated by the general course of the blood pressure and its relation to the blood supply of the abdominal organs. In amplification of this statement attention is again called to the influence which the division of different nerves exerts upon this reaction. I might also mention that an even more complete isolation of the splanchnic organs has been attempted in several instances by cutting both thoracic sympathetic, both vagi and phrenic nerves and the rami connecting the lumbar ganglia with the spinal cord. Under this condition the excitation of the central end of the thoracic sympathetic nerve gave results such as are illustrated by the accompanying figure 1. Although it is evident that

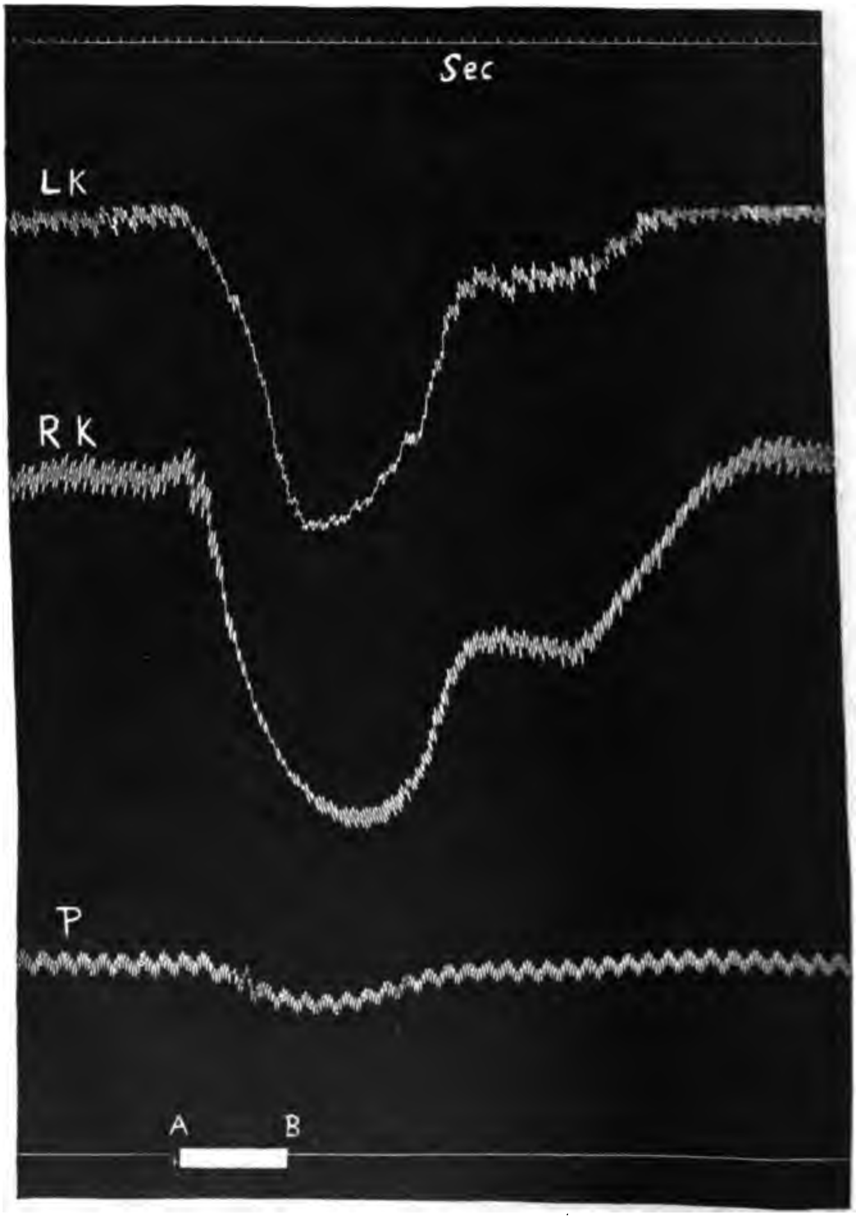

Fig. 1. Oncometric tracings of the kidneys (LK and RK) in relation with general blood pressure (P). Stimulation of the central end of the right thoracic sympathetic nerve.

the amplitude of the fall in the systemic blood pressure is somewhat diminished, its general outline is unchanged; indeed, a certain loss in its conspicuousness is to be expected, owing to the exclusion of the abdominal organs and the vascular relaxation following the division of the nerves enumerated.

As far as the vascularity of the kidneys is concerned it is apparent that it is wholly subservient to the arterial driving force. With the decline in the general pressure the volume of the kidneys decreases, while the subsequent reestablishment of normal values permits the oncometer record to attain the level previously occupied by it. The step-like adjustment of the latter, so clearly marked in this curve, corresponds to a similar accidental gradation of the blood pressure. It does not seem to have a deeper cause, because the division of the nerves precludes the possibility of an involvement of the adrenal bodies. This point, moreover, has been definitely settled by the extirpation of these organs, repeated again in the course of these experiments.

The measurements of the blood supply of the kidney by means of the stromuhr have led to results which closely agree with those just described. In illustration of this statement I insert in this place a limited number of phases which were obtained during the stimulation of the central end of the right thoracic sympathetic nerve, the left nerve, as well as all other nervous connections, having been left intact in this animal. The stromuhr (St) was inserted in the left renal vein. The pressure prevailing in this blood vessel, is indicated in figure 2 by the record V P, directly above the line of the signal marking the period of stimulation (A to B). To save space the general arterial blood pressure (AP) has been adjusted to the same zero line as the venous pressure. The data presented in the succeeding table are directly transferable to the figure.

A comparison of the values of the blood flow obtained during the stimulation with those immediately preceding and following this period, shows very clearly that the drop in the blood pressure is associated with a pronounced reduction in the blood supply of the kidney. Thus, we observe in this particular case that the normal flow of 2.59 cc. in a second is reduced, subsequent to point *A*, to 1.60 cc. in a second. It is also evident that the flow gradually increases after the cessation of the stimulation; in fact, the readjustment frequently appears even before the excitation has been completed.

These results quite agree with those obtained with the aid of the oncometer. Obviously, therefore, the division of the nerves enumerated

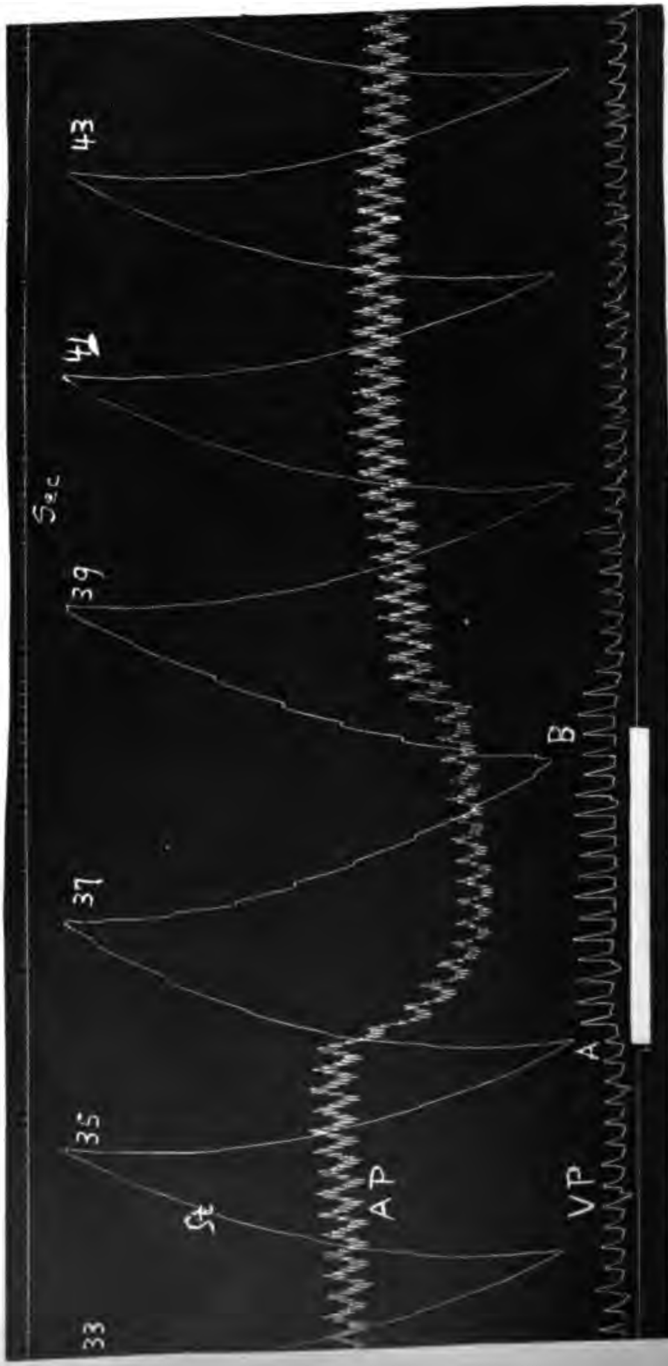

Fig. 2. The blood supply of the kidney during splanchnic depression

previously, brings it about that the kidneys meet the fall in the blood pressure in a perfectly passive manner. A slightly different picture however, is obtained, if one or both thoracic sympathetic nerves are left intact. The stimulation of one or the other of these nerves then induces a fall in the pressure which, to begin with, is characterized by a certain active resistance on the part of these organs. Very shortly, however, the loss in the arterial driving force renders this dilator attempt useless and the volume of the kidney decreases as it did when both nerves were divided. This activity on the part of the kidney betrayed itself in the curves by a slight rise in the venous pressure coincident with the early phase of the fall in pressure. The most probable explanation of this lies in a temporary augmentation of the flow through this organ occasioned by a dilation of the renal blood vessels. In general, however, it holds true that a fall in blood pressure and a diminution in the blood supply of the kidney result (a) when the intact right or left sympathetic chain is stimulated, (b) when only one of these nerves is left intact and the central end of the other is subjected to the excita-

TABLE 1

Blood supply of the left kidney

NUMBER OF PHASE	TOTAL QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD FLOW	BLOOD PRESSURE ARTERIAL	PROCEDURE
	cc.	*sec.*	*cc./sec.*	*mm. Hg.*	
29	22.4	9.2	2.43	102.5	None
30	22.8	9.1	2.50		
31	22.8	9.0	2.53		
32	22.5	9.0	2.50		
33	22.6	9.2	2.45	103.5	
34	22.0	9.3	2.36		
35	23.1	8.9	2.59	108.1	
36	23.1	9.8	2.35	67.8	Stimulation: central end, right thoracic sympathetic nerve, 25 cm., 26 seconds
37	21.8	13.6	1.60	53.0	
38	21.8	12.3	1.77	76.0	
39	23.0	9.8	2.34	82.2	
40	23.2	9.0	2.56		
41	21.7	8.1	2.67	92.5	
42	21.8	8.5	2.56	96.6	
43	22.8	8.5	2.68		
44	22.8	8.8	2.59	89.5	
45	21.0	8.6	2.44		
46	21.0	8.5	2.47	102.6	

tion and (c) when under precisely the same condition the vagi, phrenic and lumbar nerves are divided in addition to the sympathetic.

It has been brought out quite clearly by Bradford (3) that the stimulation of the distal end of the splanchnic nerve with currents of low frequency and strength gives rise to a slight fall in blood pressure which is accompanied by a shrinkage of the kidney. It is concluded by this author that this result is dependent upon a peripheral effect and not upon a reflex excitation, and hence, it must be attained with the help of certain efferent components of this nerve. It is also assumed that this shrinkage of the kidney is a passive phenomenon, made possible by dilatations in other parts of the body and especially in the intestines. Obviously, therefore, the latter organ must act as a compensatory vascular area, the relaxation of which causes a withdrawal of blood from the renal circuits and a loss in the volume of these organs.

In order to test the validity of this statement, I repeated the experiments described previously after the mesenteric arteries and veins had been ligated. As the results differ in no way from those obtained before the exclusion of the intestines, the inference seems justified that the shrinkage of the kidney is not necessarily dependent upon a dilation of the intestinal blood vessels. In fact, in accordance with the preceding statement that the fall in blood pressure persists even after the division of both thoracic sympathetic, vagi and lumbar nerves, the conclusion must be drawn that the dilatation also affects certain blood vessels which are not innervated by the splanchnic system. This reaction, therefore, is brought about reflexly through the intervention of the spinal cord and higher centers and is not due to a direct peripheral effect in the sense of Bradford. Owing to the mode of preparation, this conclusion is self evident.

In the nature of things, the only local reflex to which the reactions just mentioned could be ascribed, would be a vaso-dilation in the realm of the splanchnic organs. We have seen that the kidney is not affected in this way, and even if the blood vessels of this organ did attempt to dilate, its shrinkage is sufficient proof of the fact that the fall in the systemic pressure quite offsets the dilatation. In the second place, the results of the exclusion of the intestinal blood vessels tend to show that the volumetric decrease of the kidney is not accomplished by a compensatory transfer of blood into the vascular channels of the intestines.

This fact I have sought to establish more thoroughly by comparing the blood flow through the intestines before the excitation of the central end of the thoracic sympathetic nerve with that prevailing during the stimulation. The experimental procedure here followed was the same as that made use of in the case of the kidney. It should be mentioned, however, that the stromuhr (4) was inserted in the mesenteric vein at its junction with the vena gastro-lienalis. The venous pressure was recorded by a membrane manometer which communicated with the distal cannula of the stromuhr.

With the exception of the greater volume of the flow, these records agree perfectly with the one portrayed in figure 2. For this reason, it may suffice to insert at this time merely the values for a limited number of phases recorded during one of the stimulations. The principal fact to be gathered from this table is that the vascularity of the intestine decreases steadily with the blood pressure, the minimal flow coinciding with the lowest pressure. Obviously, therefore, the shrinkage of the kidneys is a parallel reaction and cannot be attributed to a dilation of the intestinal blood vessels. By inference it may be surmised that the other abdominal organs are affected in precisely the same way. Con-

TABLE 2

Blood supply of the intestines

NUMBER OF PHASE	TOTAL QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD FLOW	BLOOD PRESSURE ARTERIAL	PROCEDURE
	cc.	*sec.*	*cc./sec.*	*mm. Hg.*	
9	20.8	6.0	3.46	95.4	None
10	20.5	6.4	3.20		
11	20.6	6.6	3.12		
12	20.4	5.8	3.51	97.0	
13	21.4	6.0	3.56		
14	21.4	6.4	3.32		
15	20.7	6.0	3.45	96.5	
16	20.5	14.0	1.46	54.9	Stimulation: central end, right thoracic sympathetic nerve, 25 cm., 20 seconds
17	20.3	13.0	1.56	50.1	
18	20.3	9.6	2.11	58.8	
19	21.0	9.0	2.33	80.0	
20	20.0	7.6	2.63		
21	21.2	7.2	2.94	86.4	
22	21.2	6.4	3.47	98.0	
23	20.5	6.0	3.41		
24	21.0	6.2	3.38		
25	21.0	5.9	3.55	97.5	
26	20.6	6.2	3.32		

sequently, it seems that a compensation, such as has been assumed by Bradford, does not play a part in the production of this depressor reaction.

THE FUNCTION OF THE MINOR SPLANCHNIC NERVES

Asp (5) states that the stimulation of the central and peripheral ends of the divided minor splanchnic nerve leads to an increase in the blood pressure and a diminution in the frequency of the heart. These results, as will be brought out later, are not in conformity with mine; moreover, as Asp uses the singular form in his reference to the minor splanchnic nerves, it is quite probable that the wide extent of the sympathetic network of this locality had not been recognized as yet, or that he mistakes the abdominal sympathetic for the first minor. Although certain variations are present, the relatively slender abdominal sympathetic nerve most generally leaves the greater splanchnic nerve at an acute angle, and while the latter extends forward and inwards to attain the region of the supra-renal glands, the former pursues a deep course along the lumbar vertebrae, where it unites with the different spinal rami of this region. A number of ganglia are formed at these points of union, from the first three of which the minor splanchnic nerves arise. This

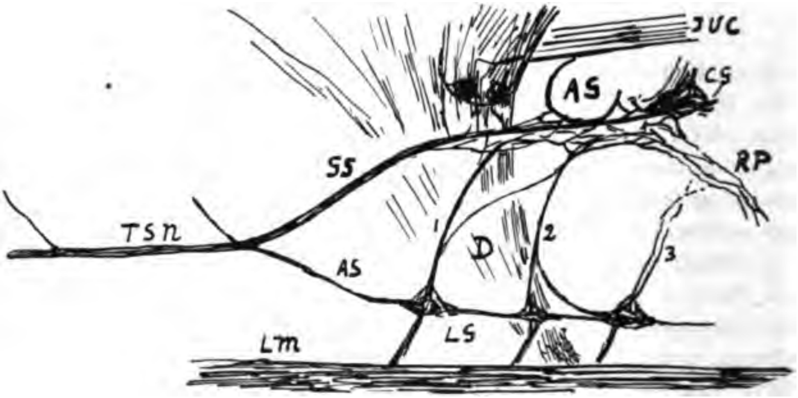

Fig. 3. *Lumbar Sympathetic System.* *TSN*, thoracic sympathetic nerve; *GS*, greater splanchnic nerve; *AS*, abdominal sympathetic nerve; *LS*, lumbar ganglia; 1, 2 and 3 minor splanchnic nerves; *RP*, renal plexus; *CG*, coeliac ganglion; *AG*, adrenal body and plexus; *IVC*, Inferior vena cava; *D*, edge of diaphragm and resected supra-renal blood vessels; *LM*, lumbar muscles.

entire system is commonly designated as the lumbar sympathetic system.

Near their places of origin the minor splanchnic nerves do not branch much and pursue a rather straight course towards the adrenal body, where they are connected by extensive ramifications with fibers of the greater splanchnic nerve. A relatively thick fiber of the latter generally continues right through this network and may be traced directly to the more distant solar ganglia. The minor splanchnic nerves, on the other hand, seem to tend largely towards the kidneys, some of their terminal fibers being directly traceable to this organ. The first and second minor nerves are frequently connected with one another by a fiber of considerable size; the second and third also show collaterals near their respective ganglia. An idea regarding the general course of the different fibers may be obtained from the neighboring sketch which gives the relationship existing on the right side of the body. To bring these nerves into view, the right kidney and liver must be pushed aside and the supra-renal blood vessels divided peripherally to the adrenal body. In a large dog the different minor splanchnic nerves may be isolated sufficiently to permit the application of the electrodes not only to the intact nerves, but also to their central or distal ends.

To begin with, the minor splanchnic nerves as well as their lumbar and thoracic connections, were left intact. The kidney of the corresponding side was placed in an oncometer, the recorder of which was adjusted in the same ordinate as the manometer registering the general blood pressure. Weak tetanic currents invariably produced a fall in blood pressure which under favorable conditions amounted to about 30 per cent of the normal pressure. The most conspicuous results were obtained with the first and second minors; moreover, these vascular depressions persisted even after the thoracic sympathetic nerve of the same side had been divided. On increasing the strength of the current, say from 25 cm. to 15 cm. distance of coils, the fall in blood pressure eventually gave way to a rise. It must be emphasized, however, that these reactions at no time attained the conspicuousness of those obtained with the help of the greater splanchnic or thoracic sympathetic nerve.

The minor splanchnic nerves were then divided about midway between the lumbar ganglia and the supra-renal plexus. The stimulations of the central end of any one of these nerves led to very characteristic depressions of the blood pressure, the amplitude of the reaction being greatest when the first minor was used and least when the third nerve was employed (fig. 4). Moreover, while the general character of the reaction could not be changed by increasing the strength of the current from 25 cm. to 15 cm. distance of coils, the resulting reflex muscular efforts tended to raise the pressure, at least at the beginning of the excitation. Curare was not employed, however, because the depressor character of these nerves could easily be established with currents of such slight intensity that perceptible muscular contractions did not result.

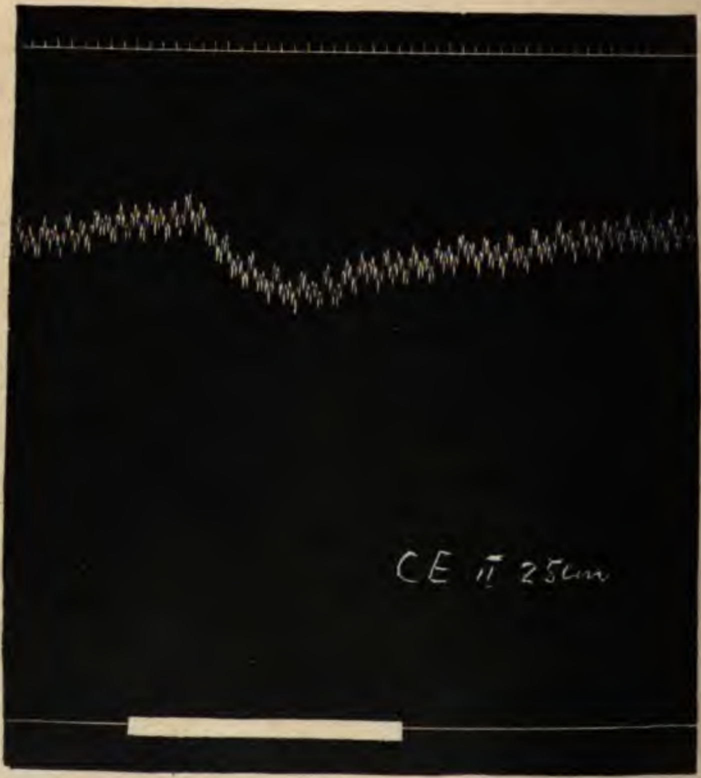

Fig. 4. Record of blood pressure during stimulation of the central end of the second minor splanchnic nerve.

These stimulations were repeated after the division of the thoracic sympathetic and vagi nerves without that a change in the character of the reaction could be noted. The cutting of the lumbar roots, however, rendered the stimulations ineffective immediately. These results clearly show that the minor splanchnic nerves conduct impulses in an afferent direction and that central parts may be attained by the latter by way of the radicles of the lumbar sympathetic ganglia. In the second place, it must be evident that the depressor action of these nerves is not dependent upon a local splanchnic reflex, but is brought about with the aid of central paths and nuclei. It is therefore a general reaction. For this reason, it cannot be regarded as similar to that of the kidney, described by Bradford as being elicited by the excitation of certain posterior roots of this locality.

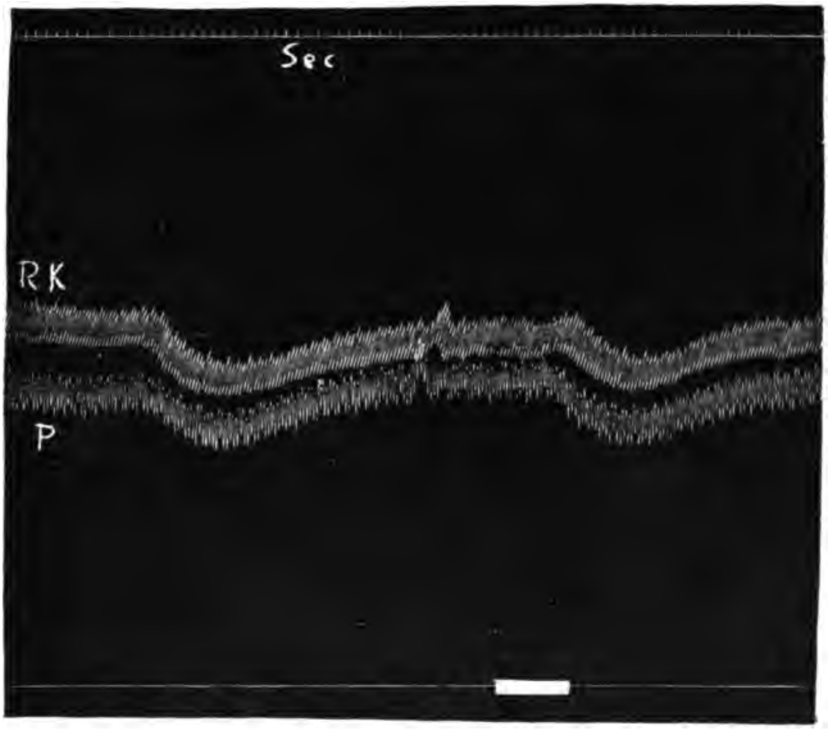

Fig. 5. General blood pressure (P) and the volume of kidney (RK) during stimulation of the central end of the first minor splanchnic nerve.

In following up this point more closely a number of oncometric records were obtained from the right kidney while the central end of the first or second minor splanchnic nerve of the same side was being stimulated. The thoracic sympathetic and vagi nerves were left intact at first but were divided subsequently. The fall in blood pressure was associated in all these cases with a diminution of the volume of the kidney; a complete passiveness of this organ, however, could only be noted after all the aforesaid nerves had been divided. A curve of the latter kind is represented in figure 5; the upper record is that of the oncometer and the lower that of the mercurial manometer. The first depression resulted in consequence of the mere adjustment of the electrodes to the central end of the divided first minor splanchnic nerve and the second in consequence of its excitation with a weak current of eight seconds duration. The parallelism between these two curves is complete, and hence, it may justly be concluded that this depressor reaction may be brought about without the aid of the kidneys. In fact, as the depression is also obtainable after the division of the nerves previously men-

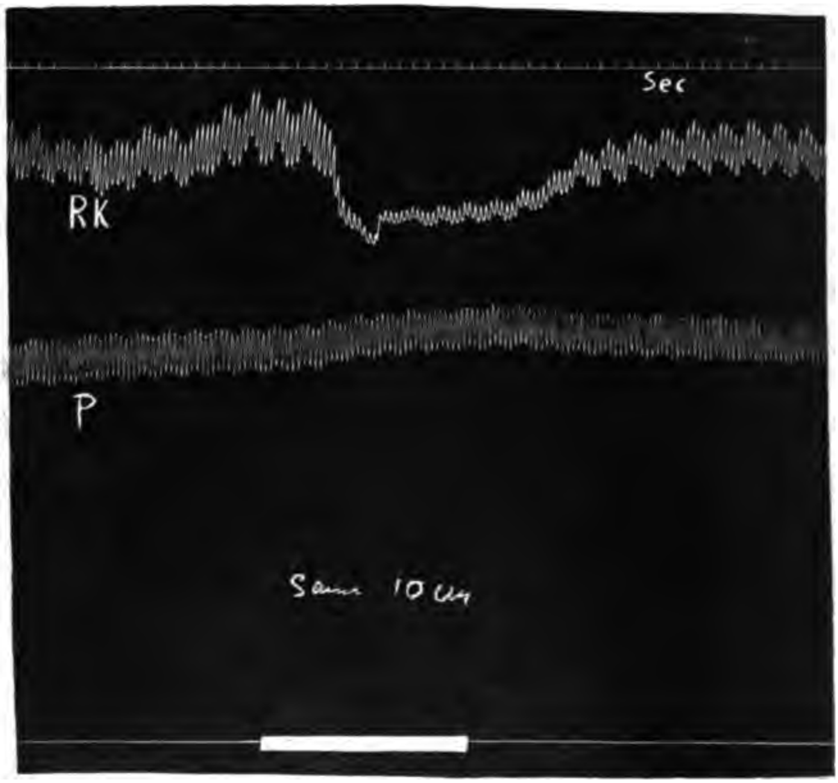

Fig. 6. General blood pressure (P) and the volume of the kidney (RK) during stimulation of the distal end of the second minor splanchnic nerve.

tioned, the splanchnic organs need not be actively implicated in this dilator process.

When tetanic currents of moderate strength were employed, the excitation of the distal end of any one of the minor splanchnic nerves resulted in a slight increase in the blood pressure. The most decisive results were obtained with the first and second nerves. A joint record of the blood pressure (P) and the volume of the kidney (RK) is given in figure 6. It may be gathered from this curve that the rise in the pressure is due to a constriction of the renal blood vessels of the same side, because if a larger vascular area were actually involved, this reaction would possess a much greater amplitude. This conclusion is borne out further by the fact that the shrinkage of the kidney continued in most cases for some time after the blood pressure had returned to its normal level. Thus, while the initial vaso-constriction in this organ tends to heighten the general blood pressure, the field affected is so small that a compensatory adjustment of the latter can readily be brought about.

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THE PHYSIOLOGY OF THE ATRIO-VENTRICULAR CONNECTION IN THE TURTLE

II. IRRITABILITY AND RHYTHM FORMATION

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INTRODUCTION

After the right and left parts of the A–V funnel of Malacoclemmys geographica had been shown to more easily conduct the excitation wave than the other parts (1), the continuation of the study of this physiological differentiation involved the investigation of a possible differentiation in irritability and in rhythm forming power. It seemed reasonable to expect that if certain portions of a muscular connection can conduct better than others, they might be more easily directly stimulated. The investigation of the rhythm forming power was suggested by the observations on ventricular rhythm described in an earlier paper (2) and the appearance of funnel rhythm in the heart of Malacoclemmys (1). It was decided also to determine the influence of the cardiac nerves on this property of the funnel fibers with particular reference to possible differences between the effects of the nerves of the right and left side.

METHODS

For testing the irritability of various portions of the heart, induction shocks were sent into it through small steel needle electrodes. Three to five pairs of these were used at one time and were stuck through the parts of the heart the irritability of which was to be tested, and so connected with switches that they could in turn be connected with a single Harvard inductorium fed by a "Franco" radio-nitrogen battery with a potential of approximately three volts. Experiments were carried out on beating (in situ and excised) and on still (sinusless) hearts. All but three of the experiments were performed on the heart of Malacoclemmys. Of these three two were made on the snapping turtle (Chelydra serpentina) and one on the painted turtle (Chrysemys picta).

THE COMPARATIVE IRRITABILITY OF THE RIGHT, LEFT AND MIDDLE PORTIONS OF THE A–V CONNECTION

Haberlandt (3) found differences between the irritability of the funnel and of the right and left parts of the base of the V in the still frog heart. His method of experimentation with single shocks is open to criticism, because it is difficult to see from the manner in which he connected the four electrodes used for stimulating, and from our knowledge of the union of the A–V funnel with the ventricle and its relation to the bulbus musculature, how he could avoid stimulating the funnel under any conditions. Nevertheless, he found that the threshold value of the funnel was lower than that of the base of the ventricle, the difference being particularly large on the right which he explains by the assumption of an escape of current to the funnel. From Nakano's results (4) on differences in conductivity of the various portions of the funnel—the right, left and dorsal portions showing this function more perfectly developed than the others—we might have expected differences also in irritability. Perhaps Haberlandt's results are to be interpreted as indicating a higher irritability of the middle portion of the funnel than that of the right and left.

In a preliminary series of experiments the irritability of various parts of the still heart was compared. Some of the results are summarized in table 1. Briefly stated they show the funnel to have a lower threshold, (as indicated by the strength of stimulus necessary to produce a contraction) for both single (quick make and break) and interrupted stimulation, than the right and left parts of the base of the V. If the funnel is stimulated lower down it is found that the stimulus necessary to produce a contraction of the V is greater. On the other hand the irritability of the A–V connection just above the A–V boundary is higher than of the portion just below.

After the funnel fibers had been thus found to be more irritable than those of the V it was possible to proceed to the investigation of the comparative irritability of the different parts of the funnel. In most of the experiments three pairs of electrodes were used. They were placed in pairs in two parallel rows across the heart just below the A–V boundary and as nearly equally spaced as possible, so that the pair on the right and left were in the right and left side of the funnel while the middle pair was half way between them. The results of these experiments are to be seen in table 2. In nearly every case the strength of stimulus necessary to produce a contraction of the still A and V, or standstill or an extra systole in the beating heart, is greater when sent into the funnel by the middle pair of electrodes than when sent in by either of the other two pairs. The differences are more evident in the still than in the beating heart. In some cases the irritability indicated by the right and left pairs of electrodes was lower than that indicated by the middle pair. It was found that by moving the outer pairs of electrodes nearer in, a higher threshold value was usually to be obtained. This led to the carrying out of a series

TABLE 1

The irritability of the A–V connection compared with that of the base of the V

	SINGLE					FARADIC				
No.	Left base of V	Right base of V	Funnel just above A–V boundary	Funnel just below A–V boundary	Funnel lower down	Left base of V	Right base of V	Funnel just above A–V boundary	Funnel just below A–V boundary	Funnel lower down
23	6.25	6.0		7.0		6.75	6.75		8.0	
24	6.5		7.75	7.50		7.0		9.0	7.75	
25	6.0	6.5		7.5	5.75	7.0	7.0		8.0	6.5
26	7.5	7.5	8.75	8.0	7.25	8.5	8.0	9.5	9.0	8.0
27	7.0	7.0		8.5	7.75	7.5	7.5		9.0	8.5
28	6.5	6.25	10.0	7.75		7.25	6.75	10.75	8.5	
29	6.0	6.5	8.0	7.5	6.0	6.5	7.25	8.5	8.25	7.25
30	5.0	5.75		8.0		6.5	7.5		8.75	
31	6.5	6.0	8.5	8.0	7.75	7.0	6.5	9.25	8.75	8.25
66	7.0		9.5	9.0		8.5		11.0	10.25	
67	6.5		7.5	6.5		7.5		8.0	6.75	
68	8.5	8.0	11.0	10.0		8.75	8.5	12.0	11.25	
69			9.5	8.0				11.25	9.5	
70			10.0	10.0	8.0			11.0	11.0	8.5
71			8.5	8.0	7.0			10.25	8.75	7.5
72			9.5	8.5	8.0			10.75	9.0	8.5

of experiments on large still hearts in which five pairs of electrodes were used. These were stuck in pairs through the right, the right middle, the middle, the left middle and the left of the base of the V just below the A–V boundary. The results of these experiments may conveniently be placed in two groups. In the first are those in which the irritability of the portions stimulated by the two outermost pairs of electrodes is highest with a gradual decrease toward the middle (table 3). In the second are those in which the irritability indicated by the outermost pairs is lowest and in which the irritability indicated by the right middle and left middle electrodes is the highest (table 4). These results lead to the assumption that in the cases given in table 3 the outside pairs of electrodes were really stuck through the right and left edges of the funnel, and that in the cases listed in table 4 these electrodes were in the base of the V outside the funnel.

TABLE 2

Comparative irritability of the left, middle and right part of the A–V connection

NO.	BEATING						STILL					
	Single			Faradic			Single			Faradic		
	Left	Middle	Right	Left	Middle	Right	Left	Middle	Right	Left	Middle	Right
32	5.5	6.0	7.0	6.0	6.5	7.0	7.25	6.0	8.75	7.5	7.5	8.75
33	5.75	5.0	7.0	6.5	5.5	7.5	7.0	6.25	8.0			
34	6.0	6.5	6.5	7.0	7.5	7.0						
35	8.25	7.0	7.5	8.25	7.25	8.0				9.0	7.5	8.5
36	7.5	6.0	7.75	8.5	6.75	7.5	8.5	6.75	7.5			
37	7.0	6.5	7.0	7.5	6.5	6.5						
38	6.0	5.5	6.25	8.0	6.5	8.5						
39	5.0	5.0	5.0	5.5	4.75	5.25	7.0	6.75	6.5	7.5	6.5	6.75
40	5.75	5.5	6.5	6.75	5.5	6.0	7.5	5.5	7.0	8.5	7.25	8.25
41	8.5	7.5	8.25	9.25	8.25	9.0						
42	6.0	6.0	6.5	6.5	6.0	7.0						
43	6.5	6.0	6.0	7.0	6.75	6.75	8.25	6.75	7.75	8.75	7.25	8.25
44	6.75	6.0	6.0	7.5	7.0	6.75						
45	6.0	5.0	7.0	5.5	5.5	6.75	6.0	4.5	6.0	8.75	6.5	9.75
46							6.5	5.5	7.0	7.5	6.5	6.75
47							4.0	3.0	6.5	6.0	4.5	7.0
49	5.75	5.5	5.5	7.0	5.0	6.0	8.75	6.0	7.5	10.5	7.5	8.75
Snapper 1	7.25	7.5	8.0	8.75	8.0	9.25						
Snapper 2	7.0	6.5	8.5	8.5	6.75	10.0						
Painted 1	6.5	5.25	6.25	6.75	6.0	7.0	8.0	6.0	8.5	11.0	7.5	10.75

TABLE 3

The comparative irritability of the A–V connection

NO.	SINGLE					FARADIC				
	Left	Left middle	Middle	Right middle	Right	Left	Left middle	Middle	Right middle	Right
56	9.25	7.5	6.75	7.5	8.75	10.75	8.25	7.5	8.25	9.5
61	7.75	6.75	6.5	6.75	7.5	9.5	7.75	7.75	8.0	8.25
62	8.0	7.5	6.75	7.75	8.5	9.5	8.5	7.5	8.5	10.0
66	7.5	7.25	6.5	7.5	7.5	9.0	7.75	7.5	7.75	9.5
69	8.5	7.5	6.75	7.0	7.75	10.0	8.25	7.75	8.0	9.75

TABLE 4

The comparative irritability of the A–V connection and of the left and right base of the V

NO.	SINGLE					FARADIC				
	Left	Left middle	Middle	Right middle	Right	Left	Left middle	Middle	Right middle	Right
58	6.0	8.25	7.25	8.75	5.75	6.75	9.5	7.5	9.25	7.0
65	5.25	8.0	6.75	7.5	5.5	6.0	10.25	7.5	10.75	6.25
67	6.5	7.5	7.0	7.25	6.5	7.5	8.5	7.75	9.0	7.25
68	6.5	7.5	8.0	7.5	6.0	7.75	9.25	8.25	8.75	7.5
75	6.25	8.75	7.0	7.5	6.5	7.0	10.5	7.75	10.75	7.0

RHYTHMIC IMPULSE FORMATION IN THE A–V CONNECTION

That rhythmic impulses due to extraneous stimulation can arise in the A–V connection of the cold blooded heart has been known for some time. Gaskell (5) observed that mechanical stimulation of the A–V ring of the still frog's heart set up a series of rapid rhythmical contractions, while stimulation of the A or of the V produced but a single contraction. Ewald (6) also found that stimulation of the funnel by piercing it with a needle resulted in a long series of contractions. Lohmann (7) pointed out that faradic stimulation of the apex of the turtle heart set up automatic contractions of the V, which were soon displaced by the Si rhythm. Also (8) that during vagal standstill one or two contractions, the impulses for which arose in the A–V connection, might appear. Gewin (9) observed, following electrical stimulation of the V of the turtle, simultaneous contractions of the V and A. And most recently Haberlandt (3) has made a study of the effects of electrical stimulation of the funnel of the frog. He found that when the needle electrodes were stuck through the funnel, there resulted a larger or smaller number of contractions which might develop into a long series. From two to twenty contractions might result from a single electric shock. Furthermore, that it was occasionally possible by a double shock to reverse for a considerable time the sequence of the beating heart, and that this might also be accomplished by the mere sticking in of the electrodes. Faradic stimulation was more effective than single shocks in the production of numerous automatic pulsations which assumed different forms and lasted for varying lengths of time. In a later paper (10) he found that faradic stimulation of the funnel of the turtle also might result in a funnel rhythm of, on the average, very short duration. Haberlandt (3) thinks that the effects of temperature in producing a rhythm of the still frog's heart are to be referred to effects on the funnel. Local warming of that alone had results, the local warming of the region just under the bulbus and of the apex being without results. By cooling the Si and at the same time warming the funnel he was able, in a few cases, to initiate a funnel rhythm.

EXPERIMENTAL

1. *Experiments on beating hearts*

a. The effects of single shocks.—Three pairs of electrodes arranged as above described were used to find out whether some part of the connection was more efficient than others in the formation of automatic rhythmic impulses. A few experiments on stimulating the still heart were made in 1912 in connection with my work on the lizard and turtle heart (2), but no conclusive results were then obtained.

Only one case of the production of a funnel rhythm following stimulation with a single induction shock has been obtained. This was in an excised heart, the funnel of which had been previously stimulated many times and by strong interrupted currents as well as single shocks. This had produced hindrances to conduction and a pulsus alternans had developed (see fig. 1). The V pulsated rapidly for about ten seconds, and then beat more slowly but irregularly, the impulses, for some of the contractions at any rate, coming from the funnel. It is impossible to say positively that the A contractions originated in the funnel, but they probably did, judging from their decreased frequency and the pause just before the Si rhythm returned (see *).

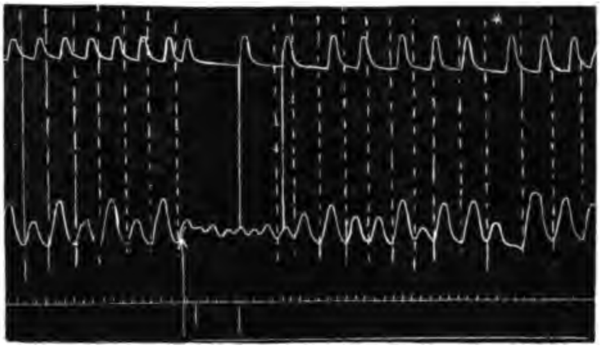

Fig. 1. "Funnel rhythm" resulting from a double induction shock to the funnel.

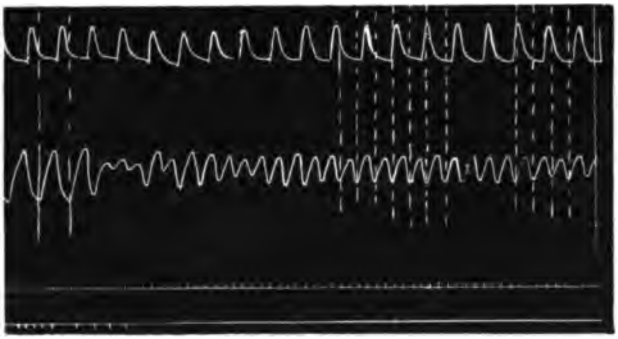

Fig. 2. Rapid V rhythm resulting from four double induction shocks to the funnel.

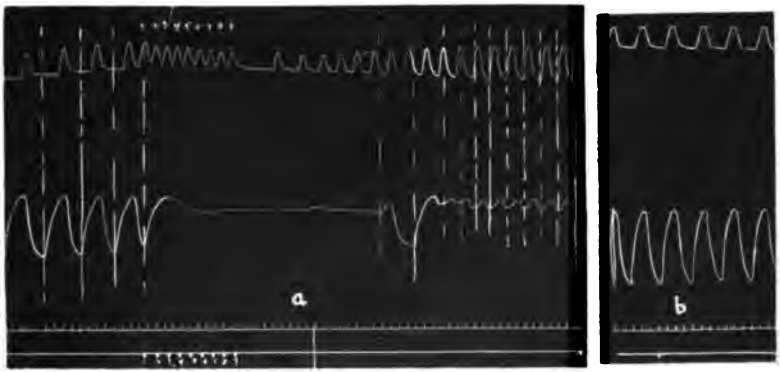

Fig. 3, *a,* V fibrillation and rapid A rhythm followed by rapid V-A rhythm resulting from nine double induction shocks to the funnel; *b,* normal Si rhythm.

Quite often when the electrodes are stuck into the funnel just below the A–V boundary there is a cessation of the rhythm for a short while, or a V fibrillation is caused which may last for a few seconds, or a rapid rhythm (flutter) which involves both V and A may be developed. But all of these are of short duration.

The effect of a series of single shocks was tried. In a few cases results of interest were obtained but only in a very few did the effects last over after the stimulation. Figure 2 shows the production in this manner of a rapid V rhythm which lasted for seven minutes, the A being unaffected and beating normally throughout. Figure 3 illustrates the production by a series of single shocks of a V fibrillation followed, after a pause, by a rapid rhythm. The A was influenced in this case, beginning to beat rapidly in response to impulses from the funnel during the V fibrillation. This rapid funnel rhythm lasted for five minutes at the end of which time it was stopped by jarring the table, being followed after a pause by the normal Si rhythm shown in the second part of the figure.

Haberlandt, as above mentioned, found that he could reverse by a single induction shock the sequence of beat of the frog's heart. He figures such a case in his figure 2. His argument (p. 17) against this being a case of retarded normal conduction is not convincing. In the first place the agreement in the time relations of the rhythm before and after the stimulation is against it as well as the appearance of block. Instead of, as he considers, having a case of a gradually wandering upward of the seat of origin of these impulses in the funnel it seems to me that we have here a case of a gradually increasing length of conduction time until block, as shown in his figure 3, appears. As he himself admits, the retarded conduction and the block are due to mechanical injury caused by the electrodes and the electric stimulation, although he found that conditions of block could be improved, and the still V be made to beat again and following the A, by stimulation of the funnel. Haberlandt found also that stimulation with a single make and break shock could produce, sometimes after a long latent period, the formation in the funnel of series of long lasting automatic impulses which began usually with incomplete and complete tetanus of the V and fibrillation, and later characterized by very frequent and regular contractions of the V but showing no influence on the A. It should be noted in referring to Haberlandt's figures 6, 7 and 8, which he gives as illustrative of these conditions, that A–V block existed or had existed. He obtained one case of this sort of thing with a sinusless heart and admits that the block in the beating heart may have been of assistance in producing these results, though not absolutely necessarily so, since he claims to have produced the same thing in a normally beating heart (see his figure 7). The heart on which this result was obtained had, however, earlier shown A–V block. Haberlandt also argues against the dependence of the formation of these effects on intracardial vagal stimulation. A glance at his figure 8 will show that there was also a negative inotropic effect on the A in this case.

b. The effects of interrupted currents. It has been found that faradic currents sent into the funnel of excised hearts may occasionally start rhythmic impulse formation there which expresses itself in one of several different forms of contraction, which, within limits, may change from one to another. Usually, however, the influence of the stimulation stops with it, the Si rhythm, after a pause, reasserting itself. Perhaps the comparatively numerous results which Haberlandt obtained on the frog's heart find their explanation in the fact that the hearts were old and the strength of the Si impulses therefore weakened. The conditions of block which he describes make this seem probable, or that the impulses could not get to the V owing to lowered conductivity in the connection. In his experiments on the turtle heart (10) he describes the results of experiments carried out on a turtle in its third day of experimentation. Be this as it may it has so far proven impossible to initiate even by the strongest interrupted currents the formation of automatic impulses in the funnel of the intact heart of Malacoclemmys. The fibrillation of the V alone, or of the A and the V, stops with the stimulation and after a longer or shorter pause the normal Si rhythm returns. Such experiments were carried out on relatively fresh hearts, never later than four hours after removal of the plastron.

The results that have been obtained with excised hearts are summarized in table 5. It should be noted that when impulses can be started in the funnel in a heart they can be started again and again, not only by stimulating the funnel, but the A and the V as well.

Usually the stimulus must be of great strength and of long duration, although occasionally a strong short stimulation has been able to start automatic impulse formation. This has been found particularly in hearts in which by previous stimulation automatic impulses have been formed, and favors the view that once the impulse formation is initiated it is easier to do so again. The results which Haberlandt reports to

TABLE 5

Summary of results obtained by stimulating the A–V connection of the beating excised heart with an interrupted current

NO.	STRENGTH OF STIMULUS	LENGTH OF STIMULUS	RESULT	DURATION	REMARKS
		seconds			
35(5)a	4.0	1	V fibrillation, followed by V rhythm after gradual slowing. A fibrillation	27 sec.	
35(5)b	2.0	0.5	V fibrillation for 5 sec. followed by V–A rhythm	33 min.	
37	3.0	9	V fibrillation, and 2 automatic V systoles	14 sec.	
37(4)	0.0	9	Rapid V rhythm of 9 contractions	18 sec.	
50(3)a	4.0	8	V standstill, followed by 2 large systoles then rapid rhythm of 6 contractions	19 sec.	Figure 6. The A contractions very weak
50(3)b	4.0	17	Rapid V rhythm. Si rate slightly increased	110 sec.	Figure 5
50(4)a	3.0	2	V standstill followed by 6 quick V contractions	13 sec.	Cf. figure 6
50(4)b	3.0	14	V standstill followed by 2 large V systoles then rapid V–A rhythm of 5 contractions	8 sec.	Cf. figure 6
78(2)a	1.0	11	V fibrillation for 5 sec. followed by irregular V–A rhythm (10 sec.), A then fibrillates followed by regular V–A rhythm for 17 seconds	32 sec.	
78(2)b	4.0	8	Rapid V rhythm, while A fibrillates	13 sec.	
78(3)a	4.0	9	Rapid V rhythm, A fibrillates, then still	16 sec.	
78(3)b	4.0	21	Rapid V rhythm, A fibrillation	24 sec.	Figure 4

TABLE 5—Continued

NO.	STRENGTH OF STIMULUS	LENGTH OF STIMULUS	RESULT	DURATION	REMARKS
		seconds			
78(3)c	2.0	24	Rapid V rhythm, A fibrillation, then still	18 sec.	
84(3)a	1.0	4	Very rapid V rhythm, A fibrillation, then still, followed by Si rhythm, slightly increased and irregular. V rhythm later irregular	8 min.	Figures 10 and 11
84(3)b	1.0	14	Very rapid, regular V rhythm, later irregular. A in Si rhythm, but irregular, suddenly V–A rhythm appears after a pause	15 min.	
84(3)c	1.0	14	Very rapid V rhythm, later irregular, then regular again, etc. A in normal Si rhythm	15 min.	
Snapper					
2(2)	2.0	1	V fibrillation (5 min.) A quickly in rapid regular rhythm from funnel impulses. V–A rhythm	65 min.	Figures 7, 8, 9, stopped by double induction shock to funnel
2(3)	3.0	2	V fibrillation (3 min.) A more rapid, then regular V–A rhythm	54 min.	
2(5)	2.0	0.5	Rapid V rhythm (15 sec.) A fibrillation (8 sec.) then still, followed by V–A rhythm	17 min.	Stopped by double induction shock to funnel

have obtained on the frog heart with threshold, or near threshold strength of stimuli, have not been substantiated, and indeed Haberlandt makes no concise claim for this on the heart of the turtle.

The automatic impulses started by faradizing the funnel of the excised beating heart exert their influence on the A and the V in a variety of forms. They may take the form of a rapid V rhythm, started dur-

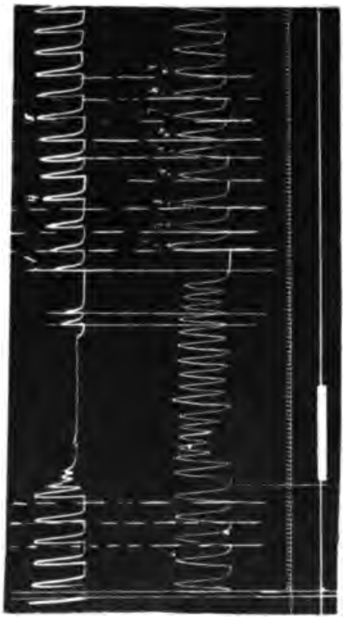

Fig. 4. Rapid V contractions and A fibrillation resulting from faradic stimulation of the funnel

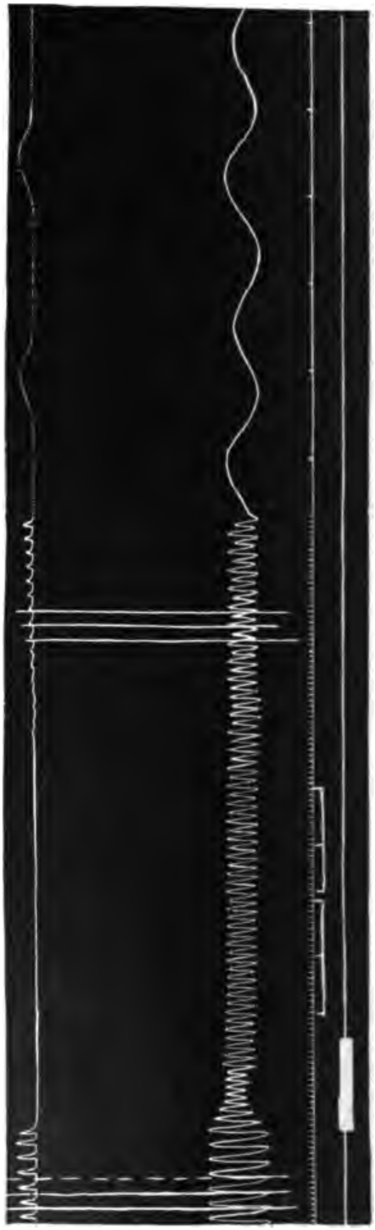

Fig. 5. Rapid V contractions, the A rate slightly increased

ing the stimulation, and consisting of separate regular pulsations which continue after the stimulation has been stopped. Figure 4 illustrates a brief case of this sort. The fibrillation of the A, as well as the negative inotropic, and perhaps also dromotropic, influence of intracardial vagal stimulation, prevents the Si impulses from reaching the funnel, thus allowing the continuation of the formation of automatic impulses there. The impulses for the two A contractions following the A fibrillation probably arose in the funnel. The formation of the funnel impulses stopped soon after this and the normal Si rhythm returned. Figure 5 shows a better example. Here the A ceases to beat (negative inotropic effect) while the V contracts rapidly, the impulses originating in the funnel continuing after the stimulation is discontinued. When the A begins to contract again, or rather when its contractions become visible again, it is seen that they are slightly more rapid than before while the V is beating almost twice as rapidly as the A. This condition lasted for a little less than two minutes, when the V paused and the Si rhythm followed.

Figure 6 illustrates how the funnel impulses for this rapid V rhythm may remain latent. The two large and the six rapid V contractions owe their origin to funnel impulses, the normal Si impulses then getting through, although they do not show on the A until later.

Faradic stimulation of the funnel may give rise to the formation there of impulses which produce a lasting over effect of V fibrillation from which various forms of rhythm may arise. The effects on the A vary. It may not be affected at all except for a slight negative inotropic effect, or its rate may be accelerated slightly. It may be brought to a standstill after a beginning fibrillation, and finally it may, after fibrillation, go over into a regular rhythmic beat which is considerably more rapid than that of the sinus, the impulses arising in the funnel. Figure 7 is a part of a typical case. This heart had been beating for some time and had previously been stimulated severely with no results. After not having been stimulated for fifteen minutes a strong interrupted current sent in for less than one second had the recorded result. Both the A and the V fibrillated, the A, however, soon beginning to beat regularly and rapidly, the impulses for the rhythm coming from the funnel. The V continued to fibrillate for about five minutes after which, after pausing momentarily, it beat as shown in the second part of figure 7, regularly, and for the most part coordinated with the A contractions, but exhibiting pulsus alternans. The pulsus alternans disappeared about five minutes later, the transi-

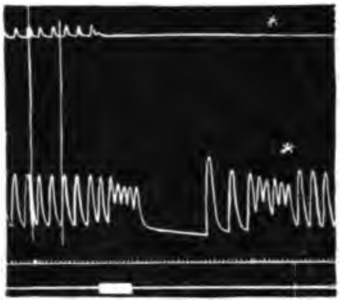

Fig. 6. Appearance of slow and rapid V contractions due to latent funnel impulses

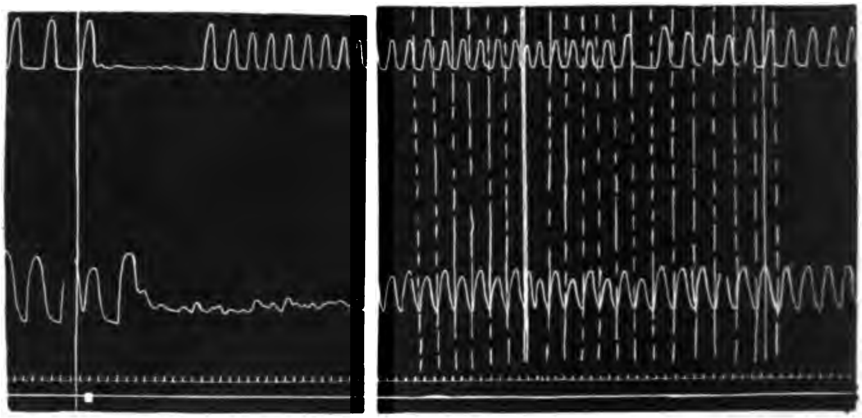

Fig. 7 *a*, V fibrillation and rapid A rhythm; *b*, V-A rhythm from funnel impulses

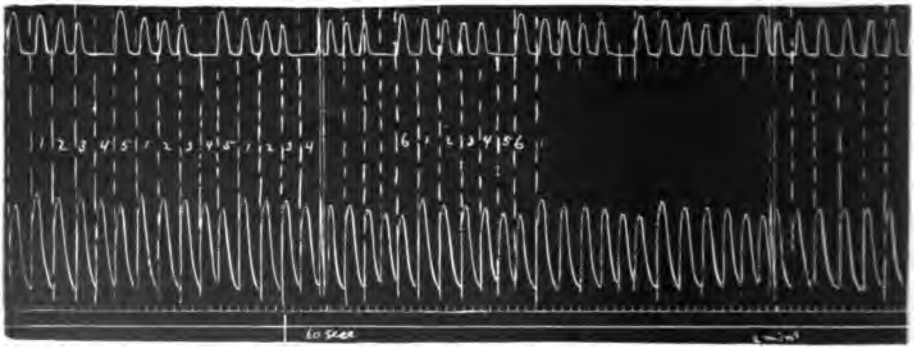

Fig. 8. Continuation of figure 7. Funnel rhythm showing V–A block

tion being shown in the figure, an As being missed after a gradual increase in vigor of contraction, following which, after a slight irregularity, the A contractions became regular and more vigorous.

Figure 8 shows a record taken of this rhythm five minutes later again. After beating regularly as shown in the last part of figure 7, a V–A block developed and the funnel impulses then arose in groups characterized by a gradual decrease in length of interval between contractions until a minimum is reached, after which there is a pause. The A–A intervals show a more rapid decrease than the V–V intervals do, and with this gradual decrease in length of interval there is a decrease in the vigor of the contractions. Beginning with systole 5, we find a maximum value between it and systole 1 for both the V and the A. This interval gradually decreases until As 4 is dropped due to refractoriness of the connection fibers, while Vs 5 follows after Vs 4 with a minimal interval. Due to the recovery in irritability, afforded by the dropping out of a beat, As 5 is most vigorous the interval being, however, greater than minimal, due, no doubt, to the weakness of the funnel impulse. After the first Vs after the pause the interval gradually decreases again and the same thing is repeated. This condition, where every fifth As is missed and where the V systoles are in groups of 5, soon improves so that every sixth As is missed and the V systoles are in groups of 6 (see middle part of figure 8). The same conditions hold here for the V as before. But when the lengths of successive A–A intervals are studied it is seen that things are a bit different. The first As after the missed beat (No. 6) still occurs in the same cycle with the Vs of minimal length of interval, while between them and the next systole (No. 1) there is the maximal length of interval which then gradually decreases with each successive cycle. The height of the V systoles also decreases regularly with the decrease in length of interval. The A systoles do so up to the last or 4th which is higher again. This fact is of interest in explaining the block and throws doubt on the assumption that the failure of the A to beat is due to the falling of the impulse into its refractory period. For if this were so it is hard to understand why the height of the last As should be more vigorous than the preceding one. There is no pause in the formation of the impulse, as the V contractions show, which would give the A fibers an opportunity to recover. It would seem that the conductivity of the funnel fibers towards the A is really decreasing from beat to beat. Through As 3 this decrease in conductivity does not become apparent owing to the gradually increasing frequency of formation of rhythmic impulses in the funnel. But between As 3 and 4, owing to this decrease in conductivity in the direction of the A, there is a slight pause (A–A) during which the A fibers recover somewhat and the next As (No. 4) is therefore more vigorous. The fifth impulse in the group finds the conductivity of the connection fibers so lowered (refractoriness of conduction) that the impulse cannot get through, while the sixth impulse owing to its weakness takes longer to reach the A again. This explanation is in keeping with that offered (1) to explain the greater vigor of the last Vs in some cases of block in which there is a gradually increasing length of interval.

The greater decrease in A–A interval as compared with the decrease in V–V interval cannot be explained by assuming a gradually wandering upward of the place of origin of the impulses in the A–V connection owing to the relation between the A and V systolic interval. A record taken two minutes later than that shown in the middle part of figure 8 shows the V–A rhythm perfectly regular and slower. If one were so minded this could now be explained as a normal A–V sequence. Figure 9, a tracing taken sixty-five minutes after the stimulation, shows this not to be the case. The rate of the V–A rhythm has slowed considerably. A single make and break shock sent into the funnel blocked the funnel impulse so that it failed to reach the A, while the V contracted once again after a pause. This shock to the funnel stopped the formation of the impulses there and gave the more slowly arising Si impulses a chance to get through to the funnel, and after this the normal Si rhythm was restored.

The fourth form of contractions which the funnel impulses may give rise to are very rapid V pulsations, the A, in most cases, continuing to beat at its normal rate. These rapid pulsations of the V have been obtained in only a single heart, but in that one three times (see No. 84, table 5). Figure 10 is an example, the return to the normal Si rhythm being shown in figure 11. The rapid V contractions soon become irregular (fibrillation) though not necessarily more rapid. The A, on the whole, beats regularly and much more slowly than the V. But its rate is usually slightly increased and there are irregularities here and there which indicate some effect on the A of the impulses arising in the funnel. Sometimes the V irregularities may disappear and reappear several times before the funnel impulses give out, and one case was obtained of a V–A rhythm developing in this way (84(3)b).

Haberlandt (3) found that the various forms of contraction set up by funnel impulses in the frog could be arranged in the following order

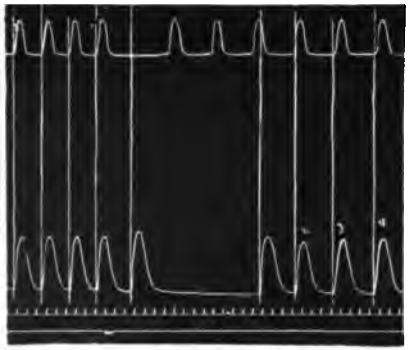

Fig. 9. Continuation of figure 8. Transition from funnel rhythm to normal Si rhythm

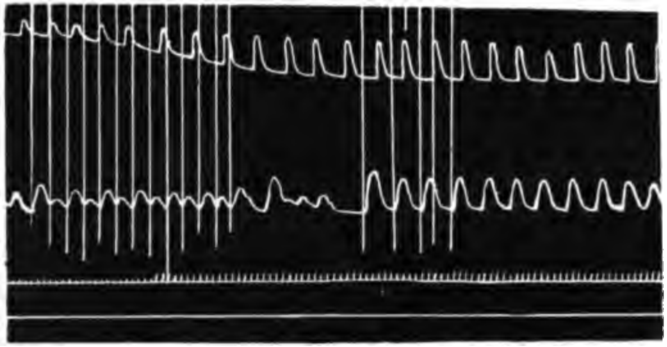

Fig. 11. Continuation of fig. 10, the giving out of the funnel impulses and the return to Si rhythm

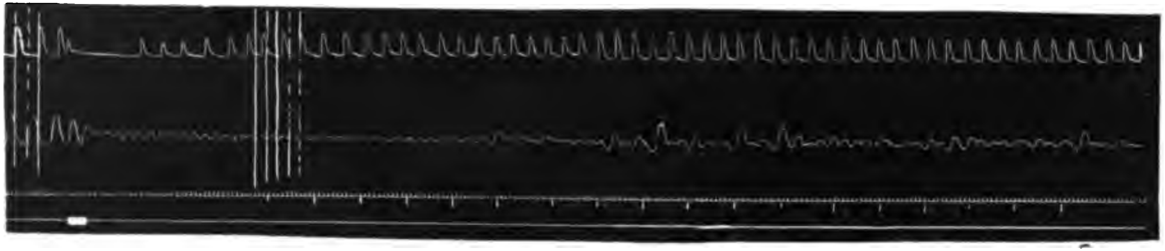

Fig. 10. Rapid V pulsations, becoming irregular, originating in funnel impulses

according to the relative ease with which they could be obtained. (1) Very frequent independent V contractions; (2) fibrillation of the V, and (3) typical atrio-ventricular rhythm. The first two never go over to the A and the rapid V contractions sometimes develop from overlasting fibrillation.

A glance at table 5 will show, notwithstanding the relative small number of cases in which it has been possible to induce automatic funnel impulses, that the most frequent form which they give rise to is a rapid V rhythm, consisting of separate pulsations which start during the stimulation and continue after it. The A is not included in the rhythm. Next in order comes typical V–A rhythm. This is, however, not a condition which arises primarily as a result of the stimulation, but follows V fibrillation or V flutter (figs. 7, 8 and 9). Next in order of sequence is a fibrillation of the V (fig. 7) which arises from a fibrillation begun by the stimulation, or from very rapid V pulsations. And finally, the very rapid V pulsations (fig. 10) which later become irregular and which may, in slowing, give rise to a V–A rhythm.

2. *Experiments on still hearts*

a. The effects of single shocks. It has not proven possible to set up a rhythm in the sinusless heart by a single make and break shock sent into the funnel. Although when the needle electrodes are put in a short rhythm may be started, the frequency of beats gradually decreasing until the formation of the impulses stops. Haberlandt (3) was able by means of a single shock to start a rhythm of a larger or smaller number of contractions, their frequency being on the average the same as the normal though sometimes much less.

b. The effects of interrupted currents. It is much easier to set up a funnel rhythm in the still heart than in the beating heart. Not that it requires a weaker current, but rather that stimulation is more often attended with success than in the beating heart. In table 6 the results of the successful cases are summarized. In several cases the funnel impulse formation would have continued longer but for the fact that it was stopped by some extraneous stimulus, such as moistening with Ringer solution, or jarring the table by removing the drum, or single or interrupted shocks. Electric stimulation was undertaken in many cases to decide whether the rhythm under observation was really proceeding from impulses formed in the funnel.

As in the beating hearts the impulses formed in the funnel may cause different kinds of results on both the A and the V. It can be stated here that the effects produced involve the A as well as the V.

In a very few cases a faradic stimulation of the funnel may result in the formation of slow rhythmic impulses which cause contractions of both the A and the V (slow V–A rhythm). Figure 12 illustrates this condition. It was obtained but twice, one of these being with a heart of the painted turtle (Chrysemys picta) where it was the only result that could be produced, being however obtainable almost at will (table 6).

A faradic stimulation may sometimes start a fairly rapid V rhythm, which as soon as the current is broken becomes a regular V–A rhythm as shown in figure 13.

The most common form of contraction to which the rhythmic funnel impulses give rise is a fibrillation of the V. This in most cases changes sooner or later into a regular, slower rhythm before the formation of the impulses stops. The A is involved, and may even begin to beat regularly during the stimulation while the V fibrillates, although in most cases it fibrillates or stands still and then, when the stimulation is stopped, begins a more or less regular rhythmic beat. The rate of the V beat is usually more rapid than that of the A and sometimes in a definite ratio to it, or the contractions of the V and A may be synchronous. Figure 14 illustrates a case of this sort. A record taken two minutes later (second part of figure) shows a regular V–A rhythm, of 2: 1 ratio. Several single shocks sent into the funnel produced merely extrasystoles of the A. Slower automatic impulses may be started in the funnel, the rhythm set up later developing irregularities which may disappear so that we have again a regular V–A rhythm. (see figs. 15 and 16).

Occasionally a very rapid rhythm is started by faradisation of the funnel. This arises from fibrillation and usually slows down until it stops. Figure 17 is a case illustrating a short-lived rhythm of this sort, the V and A beating in the ratio of 2 : 1. Figure 18 shows a more interesting case in which at first the V and A beat together at a very rapid rate. Later this rhythm slows down and becomes exceptional in that the V shows a lower rate for a time than the A. The first part of figure 19 is a record taken ten minutes later showing the incoördination between the V and the A disappearing. Forty-five minutes later the two parts are beating as shown in the middle part of figure 19 coördinatedly and in the ratio of 2 : 1, the V showing pulsus alternans. A minute later the electrodes were removed (see *). Two minutes

TABLE 6

Summary of results obtained by stimulating the A-V connection of the still heart with an interrupted current

NO.	STRENGTH OF STIMULATION	LENGTH OF STIMULATION	RESULT	DURATION	REMARKS
		sec.			
28(2)	4.0	5	V fibrillation, slow A rhythm, followed by slow V-A rhythm	90 min.	Stopped by double induction shock to funnel
28(4)	4.0	8	V-A rhythm, quick. Later V is irregular	30 min.	Started by stimulating base of V on right. Stopped by jarring
29(3)	5.0	12	Slow V-A rhythm, following fibrillation	4 min.	Started by stimulating base of V on left
29(4)	5.0	16	Slow V-A rhythm, following fibrillation	4 min.	Started by stimulating base of V on right. Stopped by faradic to funnel
29(5)a	5.0	13	Fibrillation of both A and V, ending in two quick V-A cycles	15 sec.	Started by stimulating base of V on right
29(5)b	5.0	15	Fibrillation of both A and V, ending with three quick V-A cycles	9 sec.	Started by stimulating base of V on left
36(3)	0.0	3	Slow V-A rhythm, followed by rapid and irregular V contractions, A regular, finally rapid V-A	19 min.	Figures 15, 16
36(4)	0.0	15	Fibrillation of both V and A, followed by very rapid V systoles, finally developing 2:1 V-A rhythm	5 min.	Stopped by jarring table
36(5)	4.0	13	Rapid and incoördinated V and A rhythm, later developing 2:1 V-A rhythm	3 min.	Figure 14

TABLE 6—*Continued*

NO.	STRENGTH OF STIMULATION	LENGTH OF STIMULATION	RESULT	DURATION	REMARKS
		sec.			
36(6)	0.0	9	Fibrillation of both, followed by V–A rhythm	4 min.	
36(7)	0.0	9	Fibrillation of both, followed by 2:1 V–A rhythm	5 min.	
36(8)	4.0	16	Fibrillation of both, followed by V–A rhythm	9 min.	
36(9)	2.0	11	Fibrillation of both, followed by 2:1 V–A	40 sec.	Stopped by faradic to funnel
36(10)	3.0	10	Fibrillation of both, followed by V–A	30 sec.	Stopped by faradic to funnel
39(3)	4.0	1	Slow V–A rhythm of five pulses	60 sec.	Figure 12
49(1)	1.0	11	Fibrillation of A, very rapid contractions of V, followed by V–A	9 min.	
53(2)	0.0	15	A rhythm, V fibrillation, followed by very quick V contractions giving 2:1 V–A	86 sec.	Figure 17
57(2)	2.0	15	A fibrillation, rapid V contractions, followed by V–A rhythm	90 sec.	Figure 13
61(1)	6.0	17	Rapid V contractions becoming irregular, but coördinated with A contractions	5 min.	Figures 21 and 22. Started by stimulating right auricle
81(2)	4.0	19	Very rapid V–A rhythm, gradually slowing and becoming irregular	200 min.+	Figures 18, 19 and 20
Painted turtle, (1)	3.0	1	Slow V–A rhythm of nine pulses	65 sec.	
1(2)	5.0	1	Slow V–A rhythm	3 min.	
1(3)	5.0	1	Slow V–A rhythm	7 min.	
1(4)	4.0	1	Slow V–A rhythm	5 min.	

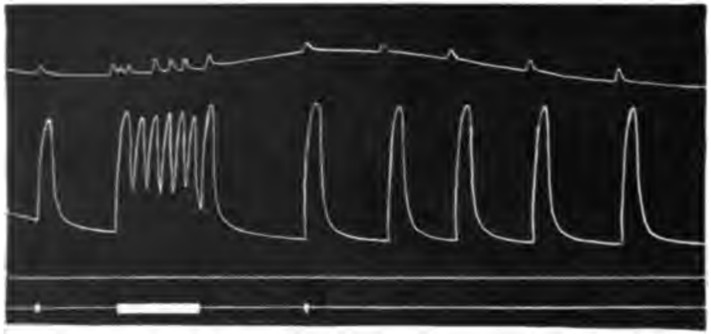

Fig. 12. Slow V–A rhythm

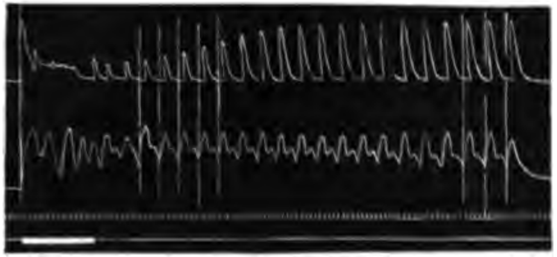

Fig. 13. V–A rhythm developing directly from rapid V contractions

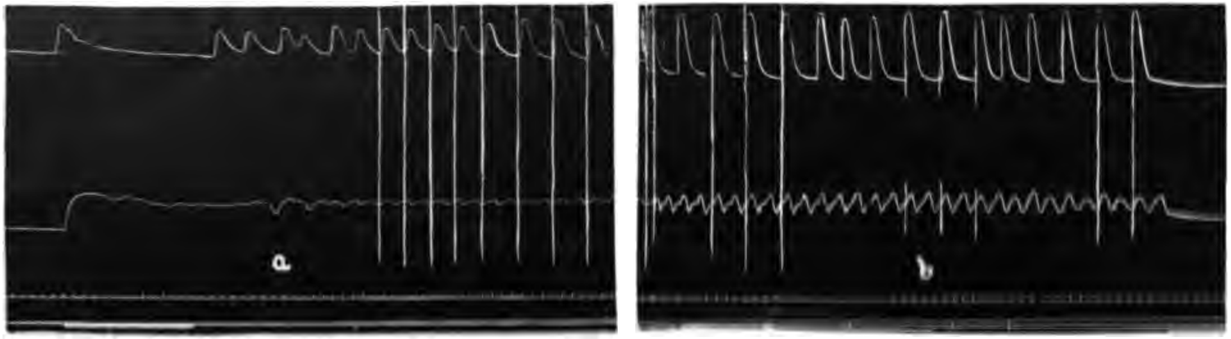

Fig. 14. The gradual development of a rapid V–A rhythm (2 : 1) after V fibrillation. Double induction shocks to funnel produce only extra systoles of the A

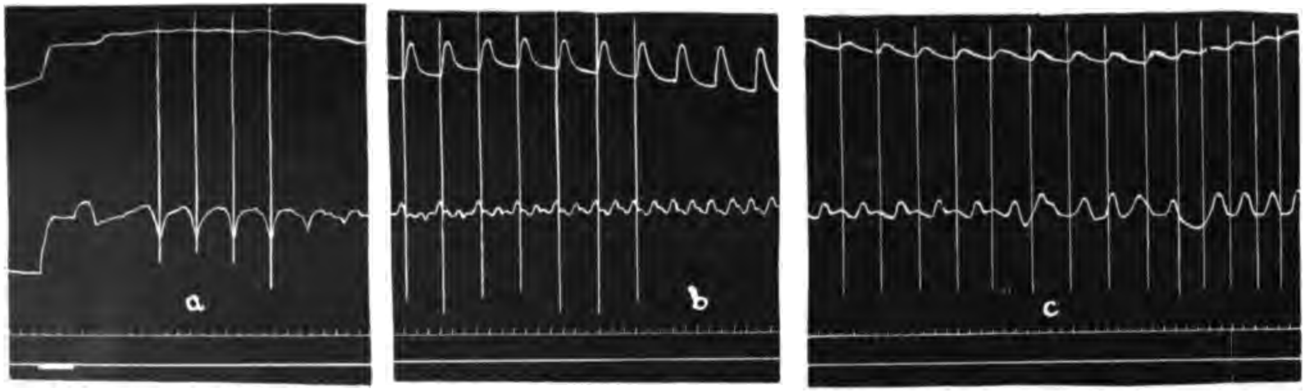

Fig. 15. Slow V-A rhythm showing V irregularities and finally becoming a regular V-A rhythm

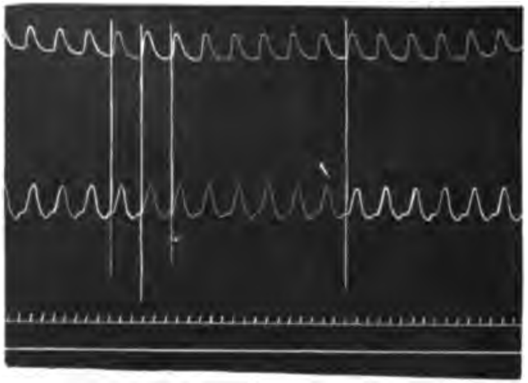

Fig. 16. Continuation of figure 15

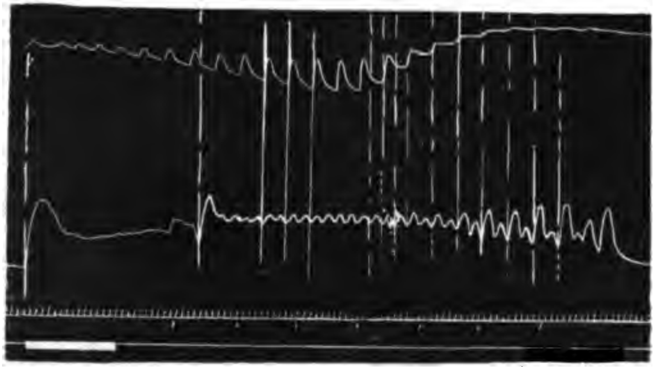

Fig. 17. Rapid V pulsations developing from V fibrillation. V-A rhythm (2 : 1)

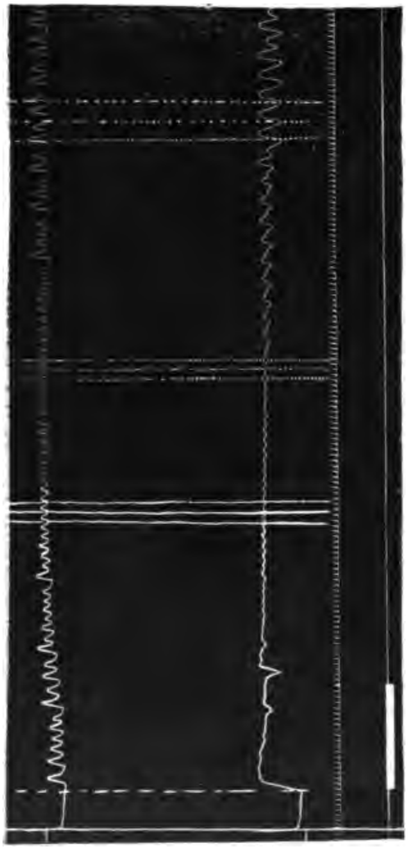

Fig. 18. Very rapid V–A rhythm developing from V fibrillation

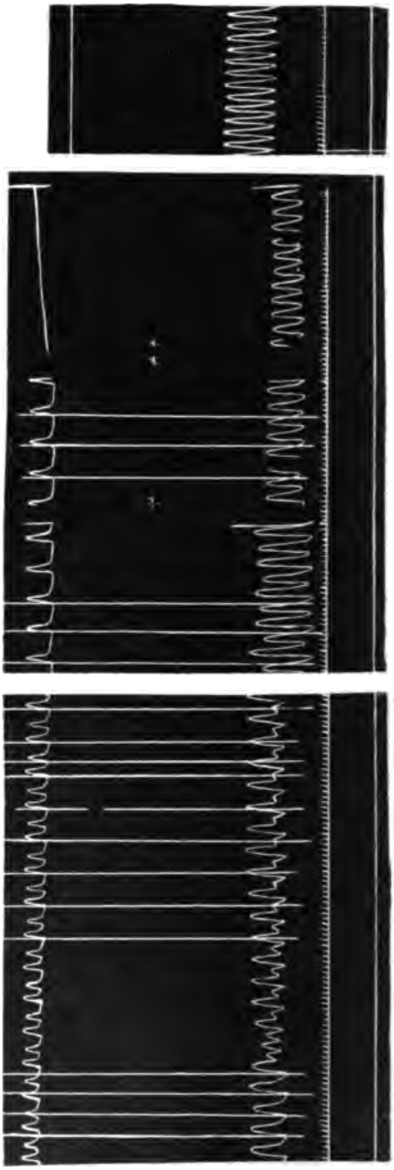

Fig. 19. Continuation of figure 18, description in text

later the A–V connection was cut through just above the base of the V (see **). The A immediately stood still while the V continued to beat. The last part of figure 19 shows a record taken seventeen minutes later (an hour and seventeen minutes after stimulation). Several attempts were made to stop this rhythm by electric stimulation (see fig. 20). At 12.52 (the stimulation having taken place at 11.15) the record shown in the second part of this figure was taken. The levers were then detached from the A and V and the two parts placed in a moist chamber. At 2.35 the V was beating between fourteen and sixteen times per minute. No further opportunity of making observations was offered until 5.25 and durng the interval the V had become still.

This "funnel rhythm" can be produced by stimulating the A or the V as well as the funnel itself. A strong current was sent into the middle portion of the funnel of a heart with the results shown in the first part of figure 21. Five minutes later the same strength of current was sent into the right auricle with the result of starting a rapid rhythm which, when the current was broken continued as a regular, rapid rhythm for about twenty-five seconds, to then become quicker and irregular, while the A began to beat more vigorously and regularly. Figure 22 is a portion of a record taken three minutes later. The V is beating irregularly and in groups, but the separate beats can be seen to be coördinated with those of the A. Soon after this the impulse formation was stopped by moistening the heart with Ringer.

In this particular case it may be a question as to where the impulse for this rhythm was being formed. Presumably in the funnel since with the strength of current used there must have been escape of current to the funnel. In several cases in which a rhythm had been started by stimulating the A in the manner just described, the effects of cutting the A–V connection were tried. Usually the rhythm stopped immediately. Occasionally, however, the V keeps on beating while the A stops. These results lead to the conclusion that, although the A was stimulated, the impulses were excited to arise not in the A but in the funnel. This matter will be referred to later.

As to the relative frequency with which the various forms of rhythmic contractions set up by automatic funnel impulses may be obtained, reference may be again made to Haberlandt for the order which he obtained in the frog. (1) The production of series of slow pulsations, (2) rapid V contractions, whose frequency is so great as not to permit of complete relaxation between them, and (3) the very frequent V contractions which may arise from overlasting fibrillation.

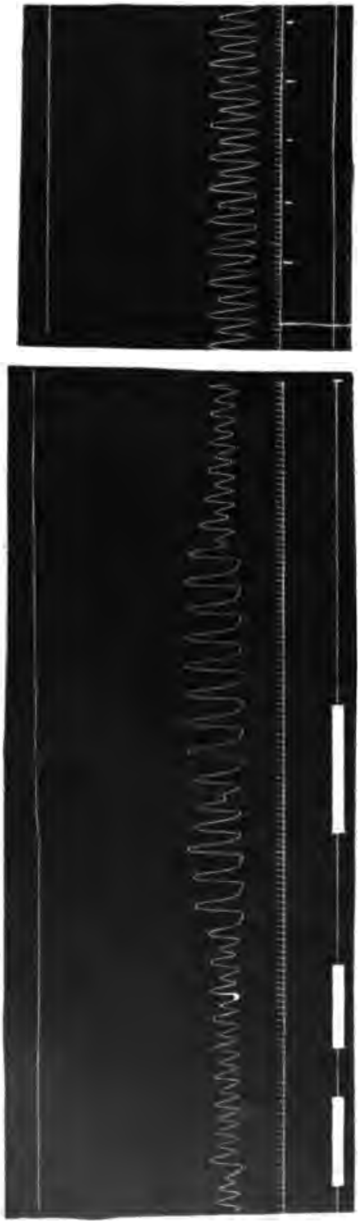

Fig. 20. Continuation of figure 19, description in text

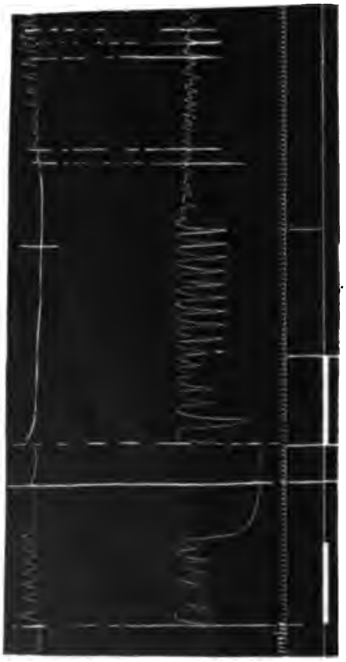

Fig. 21. Rapid V contractions arising from funnel impulses started by stimulation of the right auricle, becoming a V–A rhythm

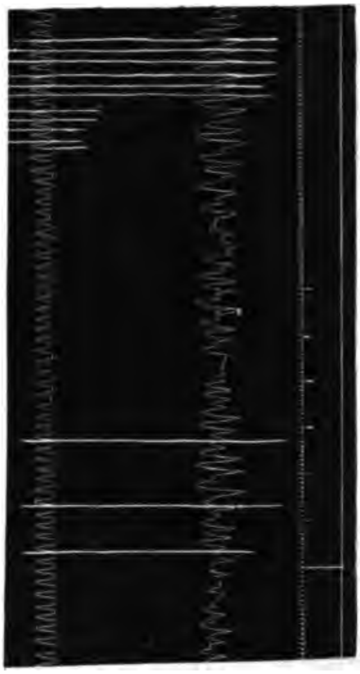

Fig. 22. Continuation of figure 21, description in text

In the still heart of Malacoclemmys the most frequent form of funnel rhythm is a fibrillation of the V which may later change into any of the others but the very slow V–A pulsations. Most frequently the fibrillation changes to a fairly rapid V–A rhythm, as in figure 14, or to a very rapid rhythm as in figures 17 and 18. Only very occasionally does the fibrillation go over into a slow V–A rhythm as in figure 15, which may arise immediately after or even during the stimulation. Sometimes the V–A rhythm develops from a rapid rhythm as in figure 13. And least frequently of all do the funnel impulses take the form of a slow V–A rhythm, as in figure 12. The A is included in all, usually coördinated with the V, and often in a 1 : 1 ratio.

DISCUSSION AND CONCLUSIONS

In addition to there being a differentiation in conductivity in the A–V connection of Malacoclemmys geographica, the same parts of the funnel, viz., the right and left, show also a differentiation in irritability, it being possible to more easily produce a contraction of the V, and of the A, by stimulating them. The differences are more clearly seen in the still heart and by using interrupted currents.

It is possible to initiate the formation of rhythmic impulses in both beating and still hearts by stimulating the funnel with interrupted currents. The impulses arise in the A–V connection and probably in the V portion of this. The rhythmic contractions which these impulses give rise to take various forms as has been described and illustrated above. It is, however, impossible to find any portion of the funnel which seems to possess this ability to give rise to automatic rhythmic impulses in greater degree than others. The strength of stimulus necessary to produce the formation of the impulses is so strong that it is impossible to contend that a certain portion only is being stimulated and that there is not an escape of current. Furthermore, stimulation of the A and of the V (apex, middle or base) can also initiate a rhythm which lasts over after the stimulation is discontinued. Haberlandt was able to show that it required stronger stimuli when the base of the V of the frog was stimulated than when the current was sent into the funnel. The rhythm set up is, however, always due to impulses arising in the funnel which is excited by the spread of the current from the place stimulated.

Single shocks are ineffective in producing a rhythm in the turtle heart except for the few cases described above, as are faradic currents of threshold or near threshold strength. Haberlandt found that he could reverse the sequence of the beat in the frog's heart by a single shock. It is difficult to understand why it is so comparatively easy to do this when the rapidly arising impulses in the funnel resulting from faradic stimulation giving rise to the characteristic contractions of the V are not also able to suppress the Si rhythm and to control the beat of the A. Haberlandt (10) cites as corroborative evidence Eckstein's results (12) on the beating of the A in normal rhythm when the frequency of the V contractions was increased by artificial stimulation. It should be remembered, however, that Eckstein also found that the V might beat more slowly than the A when the latter was artificially stimulated, and Haberlandt also observed (10) that during overlasting fibrillation of the A, the V may beat in an apparently normal frequency, the impulses for the V contractions coming from the funnel. As above shown in the still heart of Malacoclemmys the funnel impulses always go over to the A.

Haberlandt (3) reported that sometimes by electrical stimulation of the still heart an automatic rhythm may be started which has its seat of origin in the A or in the A part of the A–V connection. He cites Engelmann's results (13) and Seemann's (14) substantiation of them with the electrocardiogram, as well as Lohmann's (15) observation, in support of his contention. Engelmann's and Seemann's results are no doubt to be taken as evidence that the "funnel rhythm" may arise in the A part of the connection, and that the impulses therefore reach the A slightly before they reach the V. This is, however, still a "funnel rhythm" and not an auricular rhythm as Seemann would have us believe it is. Lohmann's results are, it seems to me, different. By suspending the Si and A, after cutting off the V, he observed in the frog and turtle that often the registered contractions of the A were faster than, and not coördinated with those of the Si. He concludes that the A is beating in a rhythm of its own. The question as to just where the pace-maker in the turtle heart is has not been until recently, if now, definitely settled. The conflicting views on this matter are reserved for later discussion, but a possible explanation may now be put forward of the results which Lohmann obtained. In all probability Lohmann suspended a part of the Si removed from the junction with the A, possibly a part of one of the veins, in which the rate of contraction (or the rate of impulse formation) was slower and in no definite ratio to that of the pace-maker, the impulses from which were setting the pace for the A contractions. This would give rise to the lack of coördination between the rates of contraction of the two parts which he was registering.

In addition to the experiments on stimulating the A directly, the results of which have been briefly referred to above, experiments were also made on stimulating the still A and V after they had been divided at the A–V boundary as close to the V as possible. It will be remembered that it was conclusively shown (1) that the still V placed in a moist chamber might, and usually did, develop a slow rhythm, and that the sinusless A did not. By stimulating the funnel of the isolated V by electrodes stuck through the middle of the base a rhythm could be started showing the various characteristics above described. When the A on the other hand was stimulated, whether at the A end of the funnel or elsewhere, this automatic rhythm did not result. Also in the cases mentioned above in which a rhythm had been started by the stimulation of the A, and in which the A–V connection was then cut, the A invariably stood still, while in a few the V continued to beat. All these negative results on the production of an A rhythm lead to the conclusion that the rhythm started in the heart has its origin in impulses arising in the A–V funnel and usually in that part below the level of the A–V boundary.

We have seen that it is impossible to produce this "funnel rhythm" in the intact heart by even the strongest stimuli, further that it can be occasionally produced in the excised beating heart, and most frequently in the still, sinusless heart. These facts make it seem probable that the failure of the funnel impulses to materialize is due to the presence of the Si impulses which assert themselves and blot out the funnel impulses. Furthermore in the beating excised hearts it is only in relatively old preparations that the funnel impulses seem to be able to last over for any length of time. The conclusion may therefore be drawn that the Si impulses must be weak, or that the Si impulses must be blocked from reaching the funnel in order that the funnel impulses should have a chance to get started. This may seemingly be done by causing the auricles to fibrillate or to stop beating, as the result of intracardial vagal stimulation (negative inotropic or even dromotropic influence), or as the result of a refractory condition of the A fibers caused by the rapid contractions. After the funnel impulses have gotten started they seem to have a better chance to continue, and thus set up a rhythm of the V. After this the A may then begin to beat, weakly at first, and either following the Si impulses or those of the funnel. It has again and again been observed when a rapid regular rhythm or fibrillation of the V has been started by faradization of the funnel, that it lasts over only so long as the A contractions (from the Si) remain suppressed. As soon as the Si impulses are able to get through to the funnel the formation of the funnel impulses is stopped.

SUMMARY

1. There is a differentiation in irritability as well as in conductivity in the A–V connection of the turtle Malacoclemmys geographica, the right and left parts having a lower threshold, as indicated by the strength of stimulus necessary to produce an impulse for contraction.

2. No part of the connection has been found to possess in higher degree than others the function of impulse formation.

3. By faradic stimulation of the connection it is possible to initiate the formation of automatic impulses there which give rise to a variety of forms of contraction. Single shocks do not have this capacity, with the exception illustrated in figure 1. A series of quick double induction shocks may, however, start the formation of impulses which produce a rapid rhythmic V beat (fig. 2) or a V fibrillation and rapid A beat followed by a V–A rhythm (fig. 3).

4. The rhythmic contractions proceeding from funnel impulses in the beating heart may take the form of:

a. Rapid V contractions in which the A does not take part (figs. 4, 5 and 6), though its frequency of beat may be slightly increased,

b. V fibrillation for a longer or shorter time, which later may develop into some other form of "funnel rhythm." The A usually fibrillates also, but not for so long a time as the V, and either stands still or begins to beat rapidly and rhythmically (fig. 7a).

c. V flutter, or very rapid but regular V contractions, the A usually continuing to beat at the normal rate though slightly irregular (fig. 10) and sometimes slightly accelerated. Occasionally the A is included in the funnel rhythm. The rapid V contractions may become very irregular and remain so up to the time of disappearance, or they may again become regular and thus alternate several times.

d. Typical V–A rhythm developing secondarily from either V fibrillation (fig. 7a), or from very rapid V contractions.

5. The rhythmic contractions proceeding from funnel impulses in the still heart may take the form of:

a. Slow V–A rhythm (fig. 12).

b. V fibrillation, while the A fibrillates, or stands still or beats in an irregular rhythm. This type of funnel rhythm may give rise to any of the others, except the slow V–A rhythm (figs. 14, 15, 17, 18).

c. Typical V–A rhythm which may develop immediately after the stimulation (fig. 13) or after fibrillation and rapid V contractions (figs. 14, 15).

d. Very rapid V–A rhythm arising almost immediately after stimulation and following a very short fibrillation (figs. 17, 18).

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BILE PIGMENT METABOLISM

VI. Bile Pigment Output Influenced by the Eck Fistula

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The Eck fistula presents an easy method by which the blood supply of the liver can be diminished, and changed from a mixed venous-arterial blood supply to a pure arterial circulation. The term Eck fistula in this paper means a free anastomosis between portal vein and vena cava together with ligation of the vena porta between the anastomosis and hilum of the liver. This operation deviates all the portal blood away from the liver into the vena cava and limits the blood supply of the liver to the hepatic artery. Dogs with an Eck fistula on a suitable diet may live a normal life for two years or longer.

It is not surprising that the Eck fistula liver is found to be smaller than normal—a decrease from the normal liver weight of 20 per cent on the average. When we recall that normally three-fourths of its blood is brought to the liver by the portal vein (Burton-Opitz (1)), the wonder is that the liver stands the operation as well as it does without showing more atrophy and degeneration. The Eck fistula liver of some months duration will show invariably a definite atrophy of the liver cells in every lobule. There is present, too, a definite fatty degeneration of the liver cells in the center of the liver lobules and some increase in the finely granular lipochrome pigment of the liver cells.

There is positive evidence that this atrophic Eck fistula liver is functionally inefficient. Phenoltetrachlorphthalein (2) injected into the blood is excreted in the bile, and under uniform conditions gives some evidence of the functional capacity of the liver. Liver poisons will cause a decreased output of this tetrachlorphthalein in proportion to the amount of liver injury. The Eck fistula liver shows a diminished capacity to excrete phenoltetrachlorphthalein—usually two-thirds or three-fourths normal capacity, after the liver atrophy and degeneration have had time to become established. We have anatomical as well as functional evidence that the Eck fistula liver is functionally below normal.

We have pointed out (3) that Eck fistula dogs with obstructed common bile ducts develop a distinct icterus, which, however, is much less intense than that observed with bile duct obstruction in a normal dog. This suggested to us that the production of bile pigments might depend in part on the functional activity of the liver parenchyma. In other words, that bile pigment formation might be in part a true synthesis due to liver cell activity rather than a passive metamorphosis of hemoglobin pigment to bile pigment depending solely on the supply of hemoglobin brought to the liver.

The experiments given below show that an Eck fistula dog with a bile fistula puts out much less bile pigment than a simple bile fistula.

EXPERIMENTAL OBSERVATIONS

The general technique of the bile fistula experiments is exactly the same as described in an earlier contribution (4). The routine care of animals and methods of collection and analysis of bile have also been described in detail. The Eck fistula operation used here was devised by Jeger (5), and consists of a lateral anastomosis between the portal and vena cava by means of special clamps. It is much like a simple entero-enterostomy done with the usual spring clamps. The portal vein is then ligated above the fistula as close to the liver as possible. These Eck fistula dogs will live a normal existence in almost every respect if kept on a limited diet. Bones and milk are important elements of this diet, and the calcium may be the most important factor. At times these dogs develop peculiar intoxications which may be fatal or at times may be warded off by the administration of calcium salts. Exercise is very important for these animals. The best results have been attained when an Eck fistula was performed first, followed after weeks or months by the bile fistula operation.

Eck fistula and bile fistula

Dog 16–15. Consult tables 31, 32 and 33.
September 13, 1915. Eck fistula operation, weight 23.5 pounds.
September 25. Dog at lowest weight, 20.5 pounds.
February 17, 1916. Simple bile fistula, weight 23.5 pounds.
Common bile duct doubly ligated but not cut. Hemoglobin 101 per cent.
February 21. Dog normal, weight 23 pounds. Hemoglobin 98 per cent.
March 3. Dog active and normal. See table 31.

March 24. Dog active and normal. Weight 20.3 pounds. Hemoglobin 51 per cent.

April 3. Dog active and normal. Weight 21.3 pounds. Hemoglobin 38 per cent.

April 10. Dog active and normal. Weight 20.8 pounds. Hemoglobin 41 per cent.

April 17. Dog active and normal. Weight 21.8 pounds. Hemoglobin 49 per cent.

April 24. Dog active and normal. See table 32.

June 19. Dog active and normal. Weight 20 pounds. Hemoglobin 78 per cent.

July 5. Dog active and normal. Weight 20.8 pounds. Hemoglobin 98 per cent. Red blood cells 6,332,000.

During summer dog was in good condition, active and strong. Usual routine collection.

September 5. Dog active and normal; usual routine. See table 33.

December 1, 1916. Dog continues in the usual condition.

TABLE 31

Eck fistula, bile fistula and bile secretion

Dog 16-15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3 4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									*lbs.*	
March 3	8	19	16	43	1.0	3.9	4.0	8.9	21.5	Urine contains trace of bile pigment
March 4				52				14.0	21.8	
March 6	19	14	15	38	3.2	5.4	6.4	15.0	21.5	
March 7	12	14	15	41	2.7	3.5	4.1	10.3	21.5	Hemoglobin 62 per cent
March 8	11	12	12	35	4.4	4.8	5.2	14.4	21.8	Stools contain no stercobilin
March 9	15	15	13	43	4.4	4.4	4.1	12.9	22.0	
March 10	13	10	10	33	4.1	4.7	6.8	15.6	22.0	
March 11				40				11.6	21.8	
March 13	7	9	7	23	1.6	2.2	2.3	6.1	22.3	Hemoglobin 45 per cent
March 14	14	18	17	49	3.2	4.0	2.8	10.0	22.0	
Average......				40				11.8	21.8	

* Eck fistula operation September 13, 1915, weight 23.5 pounds. Bile fistula operation February 17, 1916, weight 23.5 pounds.

TABLE 32

Eck fistula, bile fistula and bile secretion

Dog 16-15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3-4 hrs	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									*lbs.*	
April 24	45	19	20	84	2.3	1.3	1.8	5.4	22.0	Hemoglobin 69 per cent
April 25	23	15	15	53	3.1	3.4	1.3	7.8	22.1	
April 26	20	17	18	55	1.8	2.3	2.0	6.1	22.3	
April 27	14	14	22	50	2.2	2.8	2.0	7.0	22.5	
April 28	23	17	19	59	2.6	2.3	2.1	7.0	22.4	
April 29				36				8.8	22.5	
May 1	21	16	18	55	4.9	2.3	3.1	10.3	22.0	Hemoglobin 58 per cent
May 4	9	4	8	21	3.1	2.4	1.8	7.3	22.0	
May 5	11	16	12	39	1.2	2.2	1.9	5.3	22.3	
May 6				44				5.0	22.0	
May 8	23	20	19	62	2.6	1.4	1.3	5.3	21.5	Hemoglobin 71 per cent
May 9	15	9	6	30	0.9	0.8	0.5	2.2	21.8	
May 10	11	18	18	47	1.5	4.0	2.8	8.3	22.0	
May 11	20	6	8	34	1.0	0.6	0.4	2.0	22.5	
May 12	14	5	8	27	1.0	0.3	0.6	1.9	22.5	
May 13				28				3.8	22.5	
May 15	22	11	17	50	3.5	2.4	2.7	8.6	22.0	Hemoglobin 79 per cent
May 16	7	13	10	30	0.6	1.2	0.7	2.5	22.1	
May 17	7	8	7	22	0.4	0.6	0.5	1.5	22.8	
May 18	19	7	6	32	2.6	1.0	0.4	4.0	22.3	
May 19	12	8	13	33	2.2	1.6	3.2	7.0	22.3	
May 20				30				6.8	22.0	
May 22	22	12	8	42	4.0	1.4	0.5	5.9	21.8	Hemoglobin 67 per cent
May 23	6	6	7	19	0.4	0.7	1.0	2.1	21.8	
May 24	15	5	5	25	3.4	0.4	0.7	4.5	22.0	
May 25	12	7	5	24	4.9	2.2	0.3	7.4	21.8	
May 26	11	6	3	20	2.9	1.8	0.4	5.1	21.8	
May 27				20				5.4	21.8	
May 29	18	9	6	33	7.2	2.8	1.7	11.7	21.5	Hemoglobin 64 per cent
May 30	10	5	11	26	1.1	0.7	2.0	3.8	21.3	
June 3				70				8.2	21.0	
June 5	26	18	12	56	2.4	2.0	0.8	5.2	21.0	Hemoglobin 74 per cent
Average......				39				5.7	22.0	

* Eck fistula operation September 13, 1915, weight 23.5 pounds. Bile fistula operation February 17, 1916, weight 23.5 pounds. Urine free from bile pigments.

TABLE 33

Eck fistula, bile fistula and bile secretion

Dog 16-15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									*lbs.*	
September 5	17	10	11	38	1.9	2.3	2.9	7.1	22.0	Stools contain no stercobilin
September 6	10	8	4	22	2.0	1.6	0.7	4.3	22.5	
September 7	13	6	4	23	2.0	0.8	0.5	3.3	22.3	
September 8	11	14	10	35	3.2	3.5	2.0	8.7	22.5	
September 11	7	2	7	16	4.5	1.5	3.9	9.9	22.1	
September 12	7	5	4	16	1.2	1.0	0.6	2.8	22.0	R. B. C. 8,728,000. Hb. 103 per cent
September 13	8	6	4	18	2.5	3.2	1.9	7.6	22.5	
September 14	4	9	10	23	1.4	4.0	2.9	8.9	21.8	
September 15	8	9	8	25	0.9	1.0	0.9	2.8	22.0	
September 16				24				14.4	22.5	
September 18	6	6	6	18	2.8	6.2	4.6	13.6	22.1	
September 19	16	7	3	26	3.6	1.2	0.2	5.0	22.0	Stools contain a trace of stercobilin
September 20	8	7	3	18	5.0	6.4	1.4	12.8	22.5	R. B. C. 7,408,000. Hemoglobin 98 per cent
September 21	5	2	4	11	1.2	0.4	1.7	3.3	22.0	
Average........				22				7.5	22.2	

* Eck fistula operation September 13, 1915, weight 23.5 lbs. Bile fistula operation February 17, 1916, weight 23.5 pounds. Urine at no time contained bile pigments.

Simple bile fistula

Dog 16–146. Small, active, pugnacious, mongrel male.
April 21, 1916. Weight 18.8 pounds. Hemoglobin 114 per cent.
Simple bile fistula. Common duct ligated and cut.
May 2. Weight 17.5 pounds, hemoglobin 97 per cent. Active and normal.
May 8. Weight 17.5 pounds, hemoglobin 101 per cent. Active and normal.
May 15. See table 34.
During this time dog had no jaundice, and urine was free from bile pigments.
October 4. Dog is in good condition.

This dog (16–146) acts as control for dog 16–15 with bile and Eck fistula. Both dogs were pugnacious, active adult males of the same build but not the same breed. The control is smaller, 17 pounds as compared with 22 pounds, but puts out slightly more bile than the Eck fistula, 41 cc. as compared with the average of 39 cc. The difference in *bile pigment output* is remarkable, 17.7 mgm. for the normal as contrasted with 5.7 mgm., the average for the Eck fistula.

TABLE 34

Simple bile fistula, control for Eck fistula. Contrast tables 32 and 33

Dog 16-146*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1916									*lbs.*	
May 15	9	13	12	34	5.2	8.2	4.8	18.2	17.3	Hemoglobin 98 per cent
May 16	14	15	14	43	4.7	6.4	5.0	16.1	17.5	
May 17	12	14	14	40	4.6	5.6	5.0	15.2	17.8	
May 18	8	13	19	40	3.1	5.0	5.6	13.7	18.0	
May 19	9	15	17	41	5.1	10.0	6.8	21.9	17.8	
May 20				40				17.2	18.0	
May 22	14	15	20	49	5.4	6.0	6.8	18.2	17.8	Hemoglobin 95 per cent
May 23	8	13	15	36	4.6	6.4	6.0	17.0	18.0	
May 24	18	18	17	53	6.4	6.0	5.4	17.8	18.3	
May 25	8	17	18	43	4.0	6.8	5.7	16.5	18.1	
May 26	16	19	18	53	6.1	7.6	6.0	19.7	18.3	
May 27				32				19.4	18.0	
May 29	14	16	15	45	6.2	7.2	5.7	19.1	17.8	Hemoglobin 96 per cent
May 30	9	7	8	24	4.9	6.1	7.4	18.4	18.0	
Average......				41				17.7	17.1	

* Bile fistula operation April 21, 1916. Weight 18.8 pounds. Urine free from bile pigment.

Eck fistula and bile fistula

Dog 15–15. Strong white bull dog, female.

May 13, 1915. Weight 32 pounds, hemoglobin 91 per cent. Red blood cells 5,376,000.

Operation, Eck fistula and bile fistula done at same time. Common duct ligated and cut.

May 25. Dog in good condition, weight 30.3 pounds, hemoglobin 90 per cent. Red blood cells, 5,248,000.

May 31. See table 35.

July 15, 1915. Dog intoxicated and was sacrificed.

Autopsy. The Eck fistula is wide open, and the portal vein above it is obliterated. Bile fistula in good condition, common duct obliterated at its entrance into duodenum. Other findings not germain to this experiment.

TABLE 35

Eck fistula, bile fistula and bile secretion

Dog 15-15*

DATE	BILE: Amount in cubic centimeters				BILE: Bile pigments in milligrams				WEIGHT	REMARKS
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		Diet†
1915									lbs.	
May 31	15	15	16	46	3.1	3.4	2.9	9.4	29.5	May 13 R. B. C. 5,376,000. Hemoglobin 91 per cent
June 1	18	22	15	55	3.5	4.3	2.6	10.4	30.0	R. B. C. 5,200,000. Hemoglobin 92 per cent
June 2	13	11	16	40	1.8	2.0	2.8	6.6	29.5	
June 3	15	15	22	52	2.0	2.6	3.5	8.1	29.5	Stools contain no sterocobilin
June 7	24	22	23	69	7.5	7.2	7.3	22.0	29.3	
June 8	11	16	28	55	0.1	0.7	1.6	2.4	29.3	
June 9	20	23	18	61	3.1	6.1	5.1	14.3	30.0	
June 10	19	18	21	58	2.5	2.5	4.3	9.3	30.3	
June 11	8	10	15	33	1.3	2.7	3.6	7.6	30.0	Hemoglobin 90 per cent
June 14	29	26	16	71	2.6	3.0	2.9	8.5	29.5	Stools contain no stercobilin
Average......				54				9.8	29.6	

* Bile fistula and Eck fistula operations May 13, 1915.

† Diet of milk, bread and bones plus 50 grams liver each day. Urine contains at most a trace of bile pigments.

Eck fistula and bile fistula

Dog 16-139. Black and white mongrel male.

March 28, 1916. Weight 32.5 pounds.

Operation, Eck fistula done as usual.

April 10. Good condition, weight 30.8 pounds, hemoglobin 87 per cent.

April 24. Good condition, weight 30.8 pounds, hemoglobin 97 per cent.

April 28. Simple *bile fistula* operation.
May 16. Weight 28.8 pounds, hemoglobin 98 per cent.
May 17. See table 36.
June 12. Weight 29.5 pounds, hemoglobin 88 per cent, good condition.
July 5. Distemper developed.
July 8. Death.

Autopsy. Eck fistula is partly occluded except for three small openings, probably due to organized thrombi. The portal vein above the fistula is completely occluded. The bile passages are pale and smooth. The bile fistula is perfect, and bile is completely excluded from the duodenum. Kidneys show miliary abscesses.

TABLE 36

Eck fistula, bile fistula and bile secretion

Dog 16–139*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1916									*lbs.*	
May 17	4	10	8	22	2.2	4.3	2.6	9.1	29.0	Urine constantly free from bile pigments
May 18	8	8	10	26	3.6	3.6	4.0	11.2	28.5	
May 19	9	9	9	27	4.9	4.0	2.8	11.7	28.3	
May 20				22				14.9	28.3	
May 22	11	9	10	30	5.4	4.2	4.6	14.2	28.5	Hemoglobin 96 per cent
May 23	6	5	9	23	4.0	2.5	4.6	11.1	28.8	
May 24	9	11	10	30	2.8	4.4	3.4	10.6	29.0	
May 25	11	13	14	38	7.6	6.4	6.4	20.4	28.8	
May 26	7	9	15	31	5.7	5.2	6.8	17.7	29.1	
May 27				60				18.8	29.3	
May 29	12	10	11	33	8.7	6.0	4.8	19.5	28.8	Hemoglobin 86 per cent
May 30	8	9	8	25	2.3	3.4	3.6	9.3	29.0	
May 31	8	12	11	31	5.8	6.4	5.0	17.2	29.0	
Average......				31				14.2	28.8	

* Eck fistula operation March 28, 1916, weight 32.5 pounds. Bile fistula operation April 28, 1916, weight 31.3 pounds.

Dog 15–18. Strong, adult, Airdale female.

March 26, 1915. Weight 30 pounds: hemoglobin 110 per cent. Red blood cells, 6,340,000.

Eck fistula done as usual. *Splenectomy* and simple *bile fistula.*

Common duct ligated and cut.

March 31. Weight 29.8 pounds: hemoglobin 110 per cent. Red blood cells, 6,480,000.

April 13. See table 37.

May 4. Weight 25 pounds; hemoglobin 104 per cent; muscular tremors and intoxication.

Autopsy. The Eck fistula is wide open, and the portal vein above it obliterated. Bile is completely excluded from the duodenum, and the bile passages are smooth and pale.

The other findings do not concern this experiment.

For an accurate control of this experiment refer to Table D and many others in a preceding paper, No. III of this series, which gives the bile pigment output of a simple bile fistula plus a splenectomy.

TABLE 37

Eck fistula, bile fistula and splenectomy. Bile secretion

Dog 15–18*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Diet†
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1915									*lbs.*	
April 13	20	18	15	53	4.9	4.8	4.5	14.2	29.0	March 31, R. B. C. 6,480,000; hemoglobin 110 per cent
April 14	15	12	15	42	4.4	4.0	6.3	14.7	29.0	
April 15	23	18	21	62	7.1	5.3	7.1	19.5	29.0	Stools contain no stercobilin
April 16	27	18	19	64	9.2	5.5	5.5	20.2	28.5	
April 19	22	23	15	60	9.6	7.0	5.0	21.6	29.0	
April 20	20	22	26	68	6.9	6.7	8.7	22.3	38.8	R. B. C. 6,144,000; hemoglobin 103 per cent
April 21	22	23	20	65	7.9	8.5	6.7	23.1	28.5	
April 22	15	13	17	45	6.5	7.2	8.5	22.2	28.0	
April 23	22	19	21	62	5.9	7.4	7.5	20.8	28.5	
April 26	16	15	19	50	1.8	1.7	2.6	6.1	28.0	R. B. C. 5,367,000; hemoglobin 103 per cent
April 27	26	33	42	101	4.0	5.9	5.6	15.5	28.0	
Average......				61				18.2	28.5	

* Eck fistula, bile fistula and splenectomy operations March 26, 1916.

† Diet of milk, bread and bones plus 50 grams cooked liver each day. Urine contains at most a trace of bile pigments.

Simple bile fistula

Dog 16–5. Strong, white bull dog, male.

September 1, 1915. Weight 34.5 pounds, hemoglobin 104 per cent. Red blood cells, 5,696,000.

Simple bile fistula, common duct ligated and cut.

October 22. Weight 29.8 pounds, hemoglobin 93 per cent. Red blood cells 6,360,000. Dog is in good condition.

November 11. Weight 29 pounds, hemoglobin 111 per cent; condition is good.

January 20. See table 38.

February 5. Death from intoxication.

Autopsy. Bile fistula is perfect, and the bile passages are pale and smooth. Liver cells are slightly pigmented.

This dog serves as a control for tables 35 and 36. Other similar experiments have been tabulated in the preceding papers.

TABLE 38

Simple bile fistula, control for Eck fistula. Contrast tables 35 and 36

Dog 16–5*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Mixed diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									*lbs.*	
January 20	25	21	29	75	9.0	6.1	7.8	22.9	30.0	
January 21	22	23	21	66	7.0	8.2	8.4	23.6	31.0	Stools contain no stercobilin
January 22				32				36.5	30.5	
January 24	23	18	21	62	17.0	12.1	14.4	43.5	30.5	Hemoglobin 90 per cent
January 25	21	15	22	58	13.3	11.2	12.8	37.3	30.0	
January 26	17	22	19	58	7.2	11.4	10.	29.4	30.5	
January 27	16	17	19	52	7.9	8.0	9.4	25.3	29.5	
January 28	43	17	20	80	14.4	8.4	9.4	32.2	30.3	
January 29				54				26.8	31.0	
January 31	22	20	24	66	12.4	12.7	14.0	39.1	30.5	Hemoglobin 91 per cent
Average......				60				31.7	30.4	

* Bile fistula operation September 1, 1915. Urine contains trace of bile pigments.

TABLE 39

Simple bile fistula. Control for Eck fistula diet

Dog 15-22

DATE	BILE: Amount in cubic centimeters: 1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	Bile pigments in milligrams: 1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	WEIGHT	REMARKS: Bread, bone and milk diet
1915									*lbs.*	
November 1	15	16	18	49	13.5	15.1	16.0	44.6	31.5	Stools contain stercobilin
November 3	16	18	20	54	14.4	17.1	16.7	48.2	31.8	October 25, hemoglobin 123 per cent
November 4	17	23	20	60	16.0	19.1	16.3	51.4	31.8	
November 5	15	20	19	54	13.5	13.5	13.3	40.3	32.0	
November 6				45				41.5	31.8	
November 8	17	20	24	61	11.9	13.6	12.7	38.2	31.3	
November 9	19	23	21	63	13.3	14.2	12.8	40.3	31.3	
November 10	26	21	25	72	8.0	9.2	1 .4	29.6	31.3	
November 11	28	26	25	79	11.1	11.4	10.5	33.0	31.5	Hemoglobin 121 per cent
November 12	16	23	22	61	12.6	13.2	12.6	38.4	31.3	
November 13				55				42.5	31.3	
November 15	14	23	21	58	14.5	13.2	14.4	42.1	31.0	
November 16	18	23	20	61	15.0	18.2	12.6	45.8	31.5	
November 17	20	19	23	62	16.7	15.8	15.2	47.7	31.3	
Average......				59				41.6	31.4	

Urine contains at most a trace of bile pigments.

DISCUSSION

The experiments tabulated above are only a few out of a considerable number which have been performed. It is somewhat difficult to produce a successful Eck fistula in a dog and at the same time or later a bile fistula without impairing seriously the general condition of the animal. Both Eck fistula dogs as well as bile fistula dogs are subject to peculiar types of intoxication which are familiar to laboratory workers. It is not surprising, therefore, that dogs with combined Eck and bile fistulae are also subject to obscure and troublesome disturbances. The observations given above show the output of bile and bile pigment in dogs with bile and Eck fistulae during normal periods or at least periods of normal activity, appetite, digestion and weight. Dogs like No. 16–15, tables 31–33, are of particular interest, as this animal has been under observation with an Eck fistula for over a year in apparent perfect health.

All our experiments with combined bile and Eck fistulae (nine in all) are quite in accord, and these experiments here reported are fair examples. In all experiments there is definite evidence of a fall in bile pigment output below a normal mean—a fall sometimes to one-third normal, or perhaps at times only to three-fourths of normal, but always a fall in the average output of bile pigments per unit period under uniform conditions. There are the same daily and hourly fluctuations in bile pigment output, which we cannot at present explain. These fluctuations are present in the simple bile fistula as well as in the combined bile and Eck fistula.

It must be noted that Eck fistula dogs will do best on a diet of bread, milk and bones. This diet, rich in carbohydrates, gives a high level of bile pigment secretion, and table 39 gives a review of this factor, which was emphasized in an earlier communication (6). We must keep in mind the fact that a simple bile fistula dog on this Eck fistula diet will put out on the average more than 1 mgm. of bile pigment per pound body weight per unit period of six hours.

Splenectomy added to a combined bile and Eck fistula does not modify the picture nor does it change the general output of bile pigments. The particular experiment tabulated (table 37) gives a bile pigment secretion somewhat above the usual average, but this is not constant, and we believe has no significance.

May not the deviation of the digestion products from the portal vein into the vena cava and so around the liver be responsible for this drop in bile pigment secretion? We are unable to answer this question at present. An explanation on this basis would assume that some pre-pigment substance might be built up by the liver using materials brought to it by the portal vein. Such a substance might be used in the body or thrown out in the bile pigment depending on supply and demand. It might be assumed that with an Eck fistula this material was not as directly available, and the pigment formation correspondingly decreased. It is at least possible that some such factor is a part of this equation.

It is important to know whether the Eck fistula liver can eliminate promptly and in usual amounts the pigment radicle when hemoglobin is injected intravenously. Does the Eck fistula liver eliminate the same amount of bile pigment following an injection of hemoglobin? This question may be answered in the affirmative, but the detailed experiments will be given in a later publication. So this low pigment output can not be explained by a faulty pigment elimination by the liver. All the evidence points to faulty or abnormally low pigment formation, and this may not be limited to bile pigments. There are experiments which suggest that the hemoglobin may be involved in this incomplete pigment formation (refer to table 32 and the abstract history of dog 16–15 given above).

What is to be said about the life cycle of the red cell as calculated by the end products of hemoglobin disintegration? The pigment products are obviously much less in the Eck fistula dog. Must we assume that the abnormal environment of the Eck fistula dog tends to favor immortality among the short-lived red cells? This may suggest that the common method of computing the life of a red cell from the amount of its end products (?) is open to error. There are at least two unknowns in this equation.

We may say that the autopsy material gives no definite anatomical evidence of abnormality in the hematopoietic system. The simple bile fistula and the combined bile and Eck fistula show the same picture in the bone marrow, spleen and lymph glands. The only anatomical evidence is to be found in the atrophied Eck fistula liver which shows central fatty degeneration and atrophy. There is definite evidence that the Eck fistula liver may be functionally inefficient (2). We are inclined to explain the low bile pigment output in the Eck fistula as due to lowered functional capacity of the liver. We admit that other factors may be concerned, for example, the deviation of digestion products round the Eck fistula liver, supposing that some pre-pigment substance may be elaborated by the liver from such digestion products.

Whatever the correct explanation, the fact remains that the Eck fistula liver puts out much less bile pigment than normal under uniform conditions. This means more evidence that bile pigment can be formed independently of red blood cell or hemoglobin disintegration, which plays a part (perhaps a small part) in the secretion of bile pigments by the liver.

SUMMARY

An Eck fistula allows the portal blood to pass directly into the vena cava and excludes it from the liver.

The Eck fistula liver is atrophic with central fatty degeneration, and shows evidence of subnormal functional capacity.

The Eck fistula liver secretes much less bile pigment than the normal liver—one-third to three-fourths of normal or even less.

Splenectomy added to the combined bile and Eck fistula does not essentially modify the result.

Simple bile fistula and the combined bile and Eck fistula dogs show the same histological picture in the hematopoietic system.

This means that bile pigment formation is in part dependent upon liver function—in other words not solely dependent upon red cell disintegration. It is at least possible that the production of other body pigments (e.g., hemoglobin) may depend in part upon the constructive capacity of the liver.

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THE ORIGIN OF THE PROTEOLYTIC FERMENTS

II. Concerning the Character of Certain Ferments

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In a previous article (1) results were presented bearing on the specificity and character of the enzyme in the Abderhalden reaction and conclusions stated as follows:

1. There is an increased proteolytic activity of blood serum during pregnancy.
2. This increased activity is probably in the main due to an increase in the polyvalent ferments.
3. Normal blood serum has a weak non-specific proteolytic action.
4. The dialysis method of Abderhalden or the method of Williams and Pearce does not suffice to demonstrate a ferment developed during pregnancy acting only upon placental protein.
5. Many advanced pathological conditions give a positive reaction within the time specified for the Abderhalden test.
6. At present the Abderhalden test is quantitative, not qualitative.

Most of the work published since on the problem above stated, confirms the conclusions as given. Reviews of the literature dealing with the Abderhalden and associated reactions have recently been given by Hulton (2), Bronfenbrenner (3) and Wallis (4). Only such literature as bears directly upon the problem is noted here.

Recently Van Slyke, Vinograd-Villchur and Losee (5) using the familiar amino nitrogen method of Van Slyke, reported that normal blood serum digests placental tissue with practically the same quantitative result as the serum of pregnant women. They conclude, therefore, that in pregnancy there is no definite increase of proteolytic ferments in the serum. Consequently they question the reliability of the Abderhalden method for the diagnosis of pregnancy and also question the theory advanced by Abderhalden to explain this reaction (6). The theory is, briefly, that during gestation chorionic cells from the villi pass into the blood stream of the mother. In response to the entrance of these foreign (?) cells ferments are developed which are "protective" in character. That chorionic villi are found in the blood stream is an established fact. By some the albuminuria of pregnancy is attributed to such foreign protein. Early in the development of the Abderhalden method it was maintained, especially by the originator of the test and his associates, that such ferments were not only proteolytic but also specific. The vast amount of work done in connection with this particular phase of specificity has not served to support the contention, however. The early work upon which the test is based is thoroughly reviewed by Lake (7).

The results of Van Slyke et al. do not coincide with the generally accepted view that in pregnancy there is developed a definite increase in the concentration or strength of proteolytic ferments. Neither do they coincide with the results which I obtained and reported in the paper mentioned. Because of the apparent discrepancy in the results and the bearing which the above has on the origin of the proteolytic ferments, I repeated previous work and studied more carefully the theory of the production of the ferments in gestation.

It was found previously that the blood serum of pregnant dogs digests human placenta with the same ease as the serum of pregnant women. Consequently in this work dogs were used exclusively and the following series of experiments carried out in the order mentioned.

Series I. The action of the blood serum of normal dogs upon human placenta.

Series II. The action of the blood serum of pregnant dogs on human placenta.

Series III. The action of the blood serum of dogs injected with placental suspension.

Series IV. The action of the serum of dogs injected with split products of pepsin-trypsin digestion of human placenta.

Series V. The action of the serum of pregnant dogs injected with placental suspension.

Series VI. The effect of feeding on the amino acid content and the ferment strength of the blood serum using placenta as the substrate.

SERIES I. THE ACTION OF THE BLOOD SERUM OF NORMAL DOGS UPON HUMAN PLACENTA

Technic. Dogs were starved from eighteen to twenty-four hours. Blood was withdrawn from the external jugular vein under sterile precautions in a sterile syringe. It was at once put into a sterile test-tube with cotton stopper through which passed a safety pin. The safety pin prevented the stopper from being drawn down into the test-tube during the period of centrifuging, which was begun immediately and continued for ten to fifteen minutes at high speed. The serum was allowed to remain in contact with the solid parts of the blood until needed and was then withdrawn in a sterile pipette. The serum thus prepared was absolutely corpuscle-free in the majority of cases and its ferment strength not impaired when judged by the present method for estimating proteolytic ferments and diastatic ferments. We believe, possibly without sufficient reason, that serum so prepared more nearly approaches the normal ferment-bearing portion of the blood. Those changes which certainly must take place when blood is allowed to clot and slowly to express its serum are minimized.

Two cubic centimeters of serum was incubated with 0.5 gram of moist placenta or the equivalent of dried placenta prepared in the following manner: The placenta was obtained through the kindness of the staff of Cook County Hospital, brought to the laboratory, ground, washed and then floated off on water from a deep beaker into a flat glass dish. In this way we could inspect every particle of placental material and could exclude all tissue elements aside from the chorionic villi. This method is the only one which has proven adequate to the preparation of a white, specific substrate. Further details of the treatment are given in the previous article (loc. cit). In several of the experiments dried placenta was used. This is better suited to the method if in finely divided form and if the serum is agitated with the substrate at the beginning of incubation in order to insure a better admixture. All incubation mixtures contained 0.5 cc. to 1.0 cc. of toluol. Controls on 2 cc. of serum without substrate were run on each experiment. At various intervals the water, toluol, dialyzed iron and nitrite solution were tested after incubation and the amino nitrogen determined. Correction for materials averaged 0.20 cc. of gas. After incubation the specimens of serum and serum plus substrate were treated as given by Van Slyke et al. (loc. cit.). The disadvantages of the Van Slyke method when applied to the experiments below are apparent. Blood serum is incubated with a non-soluble, complex protein more or less finely divided, sometimes moist and always in a test-tube where uniform intermixture of serum and substrate is impossible. Again, dialysis of the products of digestion is not possible. While, therefore, the method gives very accurate results when applied to duplicate samples of the serum itself, satisfactory determinations are not always obtained when serum and substrate are incubated. This factor has made repetition of experiments necessary in not a few instances. The use of dry substrate does not overcome the uncertainty, though it does lessen it. From this it is evident that satisfactory duplicates must be run on every incubation mixture. In several instances triplicate tubes have been measured. It is possible that the reason for the uncertainty of the reaction is explained by the conclusion of Frankel (8) that ". . . . comparable results in proteolysis studies are to be obtained only when the substrates are in solution . . . ." Such discrepancy is not found when adequately tested dialyzing experiments are carefully carried out.

Experimental results. The results are calculated in milligrams of amino nitrogen in 2 cc. of serum with or without placenta.

TABLE 1

The action of the blood serum of normal dogs upon human placenta

DOG	MILLIGRAMS OF AMINO NITROGEN IN			INCUBATION TIME IN HOURS
	2 cc. serum and 0.5 grams placenta	2 cc. serum	Due to ferment action	
1	0.112	0.056	0.056	20
2	0.133	0.061	0.072	23
3	0.194	0.149	0.045	23
4	0.160	0.100	0.060	23
5	0.189	0.156	0.033	25
6	0.220	0.110	0.110	20
7	0.179	0.124	0.055	20
8	0.136	0.081	0.055	22
9	0.122	0.058	0.064	22
10	0.101	0.067	0.034	20
11	0.084	0.034	0.050	20
Average......	0.148	0.090	0.057	

The degree of digestion to be expected in a normal dog under the above conditions I believe to be fairly represented by the average figure given. For very obvious reasons there can be no absolute figure. But in selecting an average, note must be taken of the following: dogs differ in the concentration of amino acids in the blood under the same conditions; there is a rather wide variation in the ferment strength of the serum. While we have included no experiments, we have also found that the amino acids do not remain constant from day to day in the same dog, nor do the ferments. Unknown factors modify their concentration. In a few instances dogs have shown almost no ferment when a single specimen was examined but in no case was a serum found which did not contain substances giving an amino acid reaction.

SERIES II. THE ACTION OF THE BLOOD SERUM OF PREGNANT DOGS ON HUMAN PLACENTA

Technic. To compare the action of the enzymes normally present with those present during pregnancy four dogs were tested, all of which were in fine condition, and found pregnant while in the laboratory. The following reactions are typical and were performed at a time when the dogs were supposedly at practically a maximum enzyme height, i.e., a few weeks (2 to 3) previous to labor (Sloan, loc. cit.).

TABLE 2

The action of the blood serum of pregnant dogs on human placenta

DOG	MILLIGRAMS AMINO N DUE TO FERMENT ACTION	INCUBATION TIME IN HOURS
13	0.039*	24
14	0.008*	20
15	0.050*	20
27	0.062*	20

* Abderhalden dialysis tests were positive in each case.

The results agree with those of Van Slyke et al. By this method a greater concentration or action of pregnant serum over non-pregnant serum cannot be demonstrated. And yet on the other hand each of the dogs gave a *good Abderhalden test.* This the non-pregnant dog if in normal condition does not do in eighteen to twenty hours. The conclusion that the ferments of the blood are *not* increased during pregnancy must be limited *to this method.* Here by quantitative measurement the amino acid concentration in a digestion mixture is determined. In the dialysis test we measure the reaction of an indicator to substances, produced by digestion, which have passed through a semipermeable membrane out into the surrounding water. Exactly what substances react with ninhydrin has not been shown. Certainly the result is not dependent upon the amino acids entirely but is probably much influenced by the intermediate products. The micro amino method is limited to one type of product which at the present time is considered to be the ultimate unit of the protein molecule. Under the conditions of incubation the dialysis method gives opportunity for a more comprehensive reaction than does the Van Slyke. Van Slyke continued the digestion of soluble intermediate products by an acid hydrolysis but could find practically no difference in the end result. He does state, however, that pregnant sera produce an average digestion slightly greater than the normal. The variation is so marked between individual specimens, however, that the test has no practical application to pregnancy. It is important to note that there is a slight difference between the pregnant and the normal for in so large a series as that reported by Van Slyke et al. this difference cannot be attributed to experimental error which might be the case in a short series. Thar and Kotschneff (quoted by Van Slyke) report that on incubating normal and pregnant sera with placenta in dialyzing thimbles and determining the amino acids in the dialysate they obtained negative results with normal but positive results with pregnant sera. The ferment was not specific, however. When incubation experiments without dialysis were carried out there was noted no difference between pregnant and normal sera. I believe, therefore, that the method of Van Slyke is not an adequate one for testing the ferment of pregnancy in the way mentioned above and that the original dialysis technic when carefully and repeatedly controlled is of much greater value.

SERIES III. THE ACTION OF BLOOD SERUM OF DOGS INJECTED WITH PLACENTAL SUSPENSION

As mentioned earlier in this paper, the dog produces an enzyme during pregnancy which will digest human placenta within a period of eighteen to twenty hours. This digestion can be demonstrated by the dialysis technic. Furthermore, the *normal* dogs' serum does not have this power except the interval be continued for six to eight hours.

Now, if the theory presented by Abderhalden to explain this increase in proteolytic power is correct, we should expect the dog to develop a ferment when injected with chorionic cells. That the dog is not more widely used for immunizing purposes depends upon the fact that dogs are not so easily sensitized or immunized as the smaller rabbit or guinea pig. In this particular instance we should not expect such a condition to exist. Moreover, if the *method of introduction* of chorionic cells approximates the supposed method of entrance during gestation we would have much more reason to expect the generation of a ferment than if an entirely different procedure were chosen such as intramuscular injection.

Technic. Five grams of placenta which had been prepared according to the method outlined earlier was ground under sterile conditions in a small amount of salt solution until in very finely divided form. The undivided portion (about 0.5 grams) was discarded and the whole suspended in salt solution to which a little bicarbonate of soda had been added. This suspension was decidedly opalescent and very little settling-out occurred. The suspension was shaken before using, however. Four dogs were injected intraperitoneally with from 12 to 30 cc. of the suspension. Following this at intervals of three days the dogs received four to five *intravenous* doses of increasing amount (2 to 20 cc.). Blood was withdrawn and its ferment action determined as given in the table.

TABLE 3

The action of blood serum from dogs injected with placental suspension

DOG	SEX	NUMBER OF DAYS AFTER LAST INJECTION	MGM. AMINO N DUE TO FERMENTS	ABDERHALDEN TESTS	INCUBATION TIME IN HOURS
17	♀	Before	0.056		22
		10	0.082		21
		13	0.064	+ −	20
		23	0.076		20
Average...	....	....	0.069		
18	♂	Before	0.064		22
		10	0.055		21
		13	0.059	+ − −	20
Average...	....	....	0.059		
19	♀	13	0.073		20
		21	0.109		20
		7	0.059		21
		13	0.022		20
		23	0.082	−	21
Average...	....	....	0.069		
20	♀	7	0.092	−	24
		12	0.031	−	23
		26	0.066		23
		33	0.094		20
Average...	....	....	0.070		
General average		....	0.066		

[*] The Abderhalden reactions are interpreted as follows: + − questionable; − negative.

Discussion. It will be noted that the average of the results using the Van Slyke method is higher in each case than the normal given in table 1. Does this indicate an enzyme liberation? While I think that it *indicates* such, it does nothing more, since the general average is so slightly increased over the normal (0.009). Of course, a slight increase of amino acids may mean considerable increase in the total digestive products. But on the other hand when we apply the dialysis method we find no apparent increase. Consequently, the slight increase noted here is not sufficient to justify a conclusion that a proteolytic antibody or enzyme has been generated. On the other hand had the general average been 0.100 or thereabouts such a conclusion would have been justified. The results confirm those of Hulton (9) who reported experiments on the injection of human placenta into dogs subsequent to the beginning of this work. In her experiments, however, dogs were tested but once and after intraperitoneal and intramuscular injection of emulsion. No increase in ferments was noted using the Kjeldahl method for determining the degree of digestion. However, the mode of injection of the emulsion does not test the underlying theory of the pregnancy reaction as advanced by Abderhalden and his associates. It does not introduce chorionic cells directly into the circulation and in so far does not approach the supposed mechanism of production of protective ferments during gestation. Of course, antibodies are produced to many foreign materials by intraperitoneal and intramuscular introduction. But in so doing it is a question whether we introduce the foreign protein as such into the blood or whether it is not the products of the action of living proteolytic ferments which stimulate the formation of these antibodies. In the experiments above, dogs received intraperitoneal injections purely in the hope that they might the better be prepared for the intravenous injection. At the present time no preliminary dose would be given. Again, the experiments were carried over a considerable interval and the animals tested several times. Of more importance is the fact that small doses were used. Such a method approximates to a much greater degree the normal mechanism of entrance of villi and consequently more nearly approaches theoretical conditions. The results, therefore, more adequately and completely disprove the theory given by Abderhalden to explain the reaction. To further confirm the results of the somewhat uncertain Van Slyke, experiments using the dialysis method were carried out, all of which were negative. We used coagulated material for injection and to this extent theoretical conditions were disregarded. We should expect animals to react to this material if able to react to living cells.

On the other hand Wallis (loc. cit.) reports, though not in detail, experiments in which he claimed to show that heating the extract of fresh placenta to 60° destroyed the power of antigen action. The recent work of Elsesser carried out with immune sera and pure proteins is of interest along this line. He concludes (10) "The experiments have moreover shown that a rabbit may react in absolute specific manner at one time although it has not done so in a previous test and does not do so in the serum obtained subsequently" . . . . "The individual rabbits also appear to differ in their reacting powers" . . . . "The serum of an immune rabbit is not constant in its reactive power over a continued period nor in the degree of specificity it exhibits." Hulton (loc. cit.) using a different method on *pure proteins* of the same sort reports no production of ferment at all. Her method was somewhat similar to that of Van Slyke. From the work so far presented we conclude that an animal must be studied over a considerable period before any adequate conception of the reaction of that animal can be obtained. Because the results obtained above are so little in excess of the general average of normal dogs and do not show a tendency to follow an antibody curve and further because dialysis tests on the same animals and at the same time were at most questionable, I feel certain that no digestive enzymes or antibodies can be produced by coagulated, non-soluble, complex placental substance when tested in the manner given.

SERIES IV. THE ACTION OF THE SERUM OF DOGS INJECTED WITH SPLIT PRODUCTS OF PLACENTAL DIGESTION UPON HUMAN PLACENTA

Five grams of coagulated placenta was digested for twenty-four hours with a moderately strong pepsin-HCl solution. At the same time a like amount was digested by trypsin. The digestion mixtures were united, brought to a boil, filtered, neutralized with bicarbonate of soda and with 0.5 gram of finely divided placenta made up to 200 cc. with normal salt. This system would approach conditions imaginable if ferments either in the blood or in the placenta acted upon chorionic villi and caused cells and breakdown products to pass into the maternal circulation, there, or more probably in the tissue cells of specialized organs, to excite accentuation of preformed ferments or the production of new. Two dogs were injected and the results are given in the following table.

TABLE 4

The action of the serum of dogs injected with split products of placental digestion upon human placenta

DATE	PROCEDURE	MILLIGRAMS OF AMINO NITROGEN DUE TO FERMENT DIGESTION		INCUBATION IN HOURS
		Dog I	Dog II	
August 29...	Withdrew blood, injected 10 cc.*	0.036		20
September 2...	Withdrew blood, injected 6 cc.†	0.039		
September 4...	Injected 7 cc.			
September 7...	Injected 15 cc.			
September 12...	Injected 30 cc.			
September 21...	Withdrew blood	0.056‡	0.034‡	20
September 31...	Withdrew blood	0.062	0.055	20
October 5...	Withdrew blood	0.034‡	0.015‡	20

* Intraperitoneal injection.

† Intravenous injections followed.

‡ On these days Abderhalden tests were run. In each case they were *double positive or greater*. The serum gave no color at all.

Here we find as in our previous experiment that there is an indication of a ferment production in the first dog. It is merely an indication and the highest result is only 0.005 beyond the average for the normal animal: *In contradistinction to the results on the injection of coagulated protein we find here that strong Abderhaldens were obtained.* This is conclusive evidence that there occurred an increase of ferments which we were neglecting when making determinations by the Van Slyke method. Here as in pregnant animals, a strongly positive test is obtained when the serum acts upon substrate in dialyzing thimbles. The products of digestion can pass into the surrounding fluid medium. But when the incubation is carried on in a test-tube and the determination of the end result is made by the amino nitrogen quantitative technic, no such production or liberation of antibody-enzymes can be detected. Theoretically the amino nitrogen procedure should be much more delicate and therefore, more dependable. In my hands such is not the case. The results above are well within the normal variations of serum action. There is no indication of a regular, steady increase of ferments after injection of foreign protein which carry digestion to a greater degree than normal. For this reason, I do not believe that the Van Slyke method will detect the ferment of pregnancy when used under the conditions as previously mentioned. On the other hand the dialysis method with proper care will show the increase found by the majority of workers to occur during the period of gestation. Furthermore, it would seem that the theory underlying the increase of ferments during the period of intrauterine fetal life involves not so much the passage of chorionic villi into the blood stream as the passage of the digestive products of placental cells which stimulate the maternal tissue cells, possibly of special organs, to respond with a ferment of protective character. It is also possible that a weak ferment exists in the placenta itself which acts slowly upon the chorionic villi giving rise to certain substances which act as antigen.

In her recent work Hulton (loc. cit.) brought forth the suggestion that foreign protein introduced into the blood stream goes into the body cells, is hydrolyzed there, without a protein enzyme appearing in the circulating blood, concluding that hydrolysis of foreign protein is a separate action from the "fixation" of the foreign substance by the maternal body cells or the reaction of the body to these cells. The results presented above might be so interpreted but only if the interpretation be limited to the method of Van Slyke. On the other hand, based on the dialysis method, no such interpretation of the results can be made. In other words, it would seem reasonable to suppose that in the great majority of cases antibodies or enzymes are produced as a response to the injection of foreign protein and that these antibodies appear in the blood stream but in certain instances the methods are not sufficiently delicate to detect them. Hektoen and Carlson (11) stated in their work that blood cells of foreign origin when introduced into the blood stream of another animal very quickly disappeared but that antibodies were produced which could be shown to be present not only in the blood stream but also in the other circulating fluids. They state, "Our results consequently support the view that antibodies in active immunization are produced outside the circulating blood into which they find their way at the end of the latent period." This it would seem is applicable in the great majority of cases to the introduction of foreign protein. It is very probably applicable to the injection of placental protein but because the detection of ferments is more difficult than the detection of lytic agents to blood corpuscles it is less apparent under certain conditions. It would seem to us that the method of Van Slyke is not one to be used to determine the presence or absence of such antibodies. Based on the above results and those of Thar and Kotschneff and Elsesser, I believe that dialysis plays a very important rôle in the discrepancy between the so-called Abderhalden dialysis test and that of Van Slyke.

SERIES V. THE ACTION OF THE SERUM OF PREGNANT DOGS INJECTED WITH PLACENTAL SUSPENSION

Two dogs known to be pregnant were injected with placental suspension in much the same manner shown in the experiments reported above. Serum was removed in the same manner and the digestive action upon placenta determined by the Van Slyke method. Neither dog showed any increase of proteolytic ferments. The variations were not less than normal nor the limits greater. Consequently, based on this method, the conclusion is reached that the pregnant dog is no more able than the non-pregnant to mobilize or generate enzymes capable of digesting placental suspension. In this connection it is interesting to note that neither dog aborted as a result of the injections. At the conclusion of the experiment one dog was injected with the products of the action of serum (on placenta) from animals which had received intravenous placental injections. Five pups were born twenty-four hours later and the mother died at the end of about forty-eight hours. Three pups had not been delivered. The uterus had ruptured and the head of one pup protruded through the rent. We have not known of a dog dying of a ruptured uterus and present this purely as an interesting observation. The pups delivered were at term. What effect the injection had is a question.

SERIES VI. THE EFFECT OF FEEDING ON THE AMINO ACID CONTENT AND THE FERMENT STRENGTH OF THE BLOOD SERUM USING PLACENTA AS SUBSTRATE

It was thought valuable to observe the effect of feeding upon the amino acid content and the ferment strength of the serum in animals under the ordinary laboratory treatment. We did not desire to determine such on animals under constant diet, for such a procedure would have little bearing upon conditions ordinarily met with while using the laboratory animal. Ross (12) in this laboratory has reported carefully conducted experiments on the increase in amino acids one-half hour and four hours after feeding dogs one pound of ground lean meat. After one-half hour he finds no changes but four hours after feeding the amino acids are quite considerably increased. This increase is much greater than the increase observed on the less protein diet of the ordinary laboratory animal.

TABLE 5

The effect of feeding on the amino acid content and the ferment strength of the blood serum using placenta as substrate

EXPERIMENT	INTERVAL AFTER FEEDING	SERUM	SERUM	SERUM AND PLACENTA	SERUM AND PLACENTA
	min.	*before*	*after*	*before*	*after*
1	30	0.054	0.091	0.131	0.123
2	30	0.075	0.075	0.112	0.118
3	120	0.130	0.138	0.191	0.171
4	150	0.124	0.124	0.149	0.149
5	165	0.099	0.104	0.115	0.115
6	240	0.108	0.102	0.148	0.148
7	300	0.076	0.094	0.124	0.124
Average......	....	0.094	0.104	0.138	0.135

Due to feeding 0.010
Due to ferment action 0.044

Here we note a slight increase in the general average of amino acids when animals are fed upon the ordinary mixed food of the laboratory. On the other hand there is practically no change in the enzymes. It is possible that the latter is due to the inhibition of ferments by the increase in the lipoidal content of the serum.

CONCLUSIONS

1. Normal blood serum contains a weak proteolytic ferment which carries digestion to the amino acid stage. The concentration of this ferment is not constant.
2. There is an increased proteolytic ferment action of serum during pregnancy as shown by the dialysis method of Abderhalden. This cannot be detected by the Van Slyke method.
3. The injection of placental suspension does not give rise to an increased production or mobilization of ferments when tested by the dialysis method or the Van Slyke method.
4. The injection of the split products of pepsin-trypsin digestion of human placenta gives rise to an increase in the ferments. This increase can be shown by the dialysis method but not by means of the Van Slyke method.
5. The theory underlying the Abderhalden reaction as advanced by Abderhalden does not explain the increase in ferments found during

pregnancy. The basis of the theory would seem to be the entrance of split products of placental hydrolysis rather than of chorionic villi and cells.

6. The Van Slyke method is not applicable to determinations of the above character in which a non-soluble, moist, complex, protein substance is incubated with serum in a test-tube but without dialysis.

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THE INFLUENCE OF PARTIAL THYROIDECTOMY IN PIGS

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INTRODUCTION

The work reported in this paper was undertaken at the suggestion of Dr. A. J. Carlson, with the view of determining if cretinism can be produced in this animal.

It is difficult to carry out complete thyroidectomy in the pig, owing to the fact that considerable accessory thyroid tissue is scattered along the entire cervical region, and is present in the thoracic cavity of some individuals.

Although there is a vast amount of literature available concerning thyroidectomy in experimental animals, that pertaining to the pig is very meager.

Horsley (1) in 1886, reported that removal of the thyroid in two pigs was followed by symptoms of tremor, leucocytosis and anemia with subnormal temperature. In 1892 (2) he reported that experiments by himself and Munk yielded negative results, but that the animals were not observed for a sufficient length of time. He leaves the impression that this group of pigs would have very likely developed the characteristic symptoms and died had they been observed longer. He also states that the results are much more serious in young animals, and that compensatory hypertrophy takes place very readily in young animals. He classifies the pig (with the sheep, goat and donkey) in the group of animals which slowly develop general cachexia, with death after thyroidectomy.

Eiselberg (3) removed the thyroid from a pig four weeks of age. He reports a marked retardation in growth, unaccompanied, however, by apathetic idiocy, the animal being as lively as normal animals.

Moussu (4) operated upon one pig fifteen days of age. It remained normal until about one month later when it began to show symptoms of myxedema. It became sickly in appearance, the skin became bald in places, and in others was covered with remarkably long and thick bristles. There was swelling of the abdomen and formation of infiltrated cutaneous folds. There was also retarded growth. The animal died at the age of two months and four days. Post-mortem symptoms were stated to be similar to those found in human cretins.

The thyroidectomized and control pigs. From three pigs the thyroid was removed by the operation described below. Three other pigs were kept as controls. The six pigs were from the same litter of seven. The mother, a grade white sow was immune to cholera, and on April 15, 1915, gave birth to seven healthy strong pigs. In the litter were two females and five males. One female and two males were operated upon, and one female and two males were used as checks. The operated pigs consisted of a black and white male, a white male and a white female. The checks consisted of a black and white female and two white males.

The pigs, with their mother, were kept in a warm, clean box stall, and on bright sunshiny days enjoyed the freedom of an outside pen.

When the pigs were eight weeks of age, they were weaned, and then placed in pens along with the university herd. At first, the operated pigs were placed in one pen and the checks in another, and later placed all together in a large pen with an open yard adjoining. They were well cared for and given all they would eat of a properly balanced ration.

When four months of age the pigs were given a protective dose of hog cholera serum. Hog cholera existed on the farm and the pigs were vaccinated as a matter of precaution.

In November the pigs were placed in one of the hog houses of the serum plant. They were placed in a large inside pen and were well fed and taken care of. They were in a building which was used for quartering hog cholera virus pigs, and accordingly were carefully watched for any symptoms of cholera, but as none developed they were thought to be immune from the disease.

Anatomy of the thyroid. In the pig the thyroid consists of two lobes, closely attached to each other by means of connective tissue, so that an isthmus cannot be distinguished. The gland varies slightly in position in different individuals, but is always situated on the ventral surface of the trachea, and usually a couple of inches below the larynx, but may be in contact with it. Sisson (5) states that in the adult the lateral lobes are irregularly triangular in outline and in a large adult are two inches or more in length; and that the chief artery enters at the posterior extremity.

In the week old pig the gland is similar in general arrangement to that of the adult. Each lobe is thin, rather ovoid in shape, and about the size of a bean. The two lobes are intimately connected, except at the posterior end where each lobe is pointed. The gland varies in size, but on an average weighs about 0.5 gram. On section the alveoli are about twice the size of those in an adult normal hog.

The parathyroids in this animal, according to Ellenberger (6) differ from those found in other animals in that the median pair are missing. He further states that the lateral parathyroids in ruminants and pigs do not extend to the thyroid, but remain permanently at the cranial end of the thymus; and that in mature animals as the thymus disappears they are surrounded by a small amount of thymus tissue or fat. If the position of the lateral parathyroids can be in any way judged by the position of the thymus, they are quite apt to vary in position. Sisson (5) states that the thymus in the pig may extend up the cervical region to a varying extent, that it may extend to the larynx or even to the mandibular space. We have found the position of the thymus in the fetus to vary, but it is usually found at or near the thoracic inlet. Owing to the location of the parathyroids in this animal there is not much danger of removing them in a thyroidectomy; and the symptoms following such an operation should not be accompanied by the symptoms following a parathyroidectomy.

The presence of an accessory thyroid, somewhat oval in shape and about the size of a marble is quite constant at the thoracic inlet.

The operation. The three pigs were thyroidectomized aseptically and under ether anesthesia when eight, twelve and fifteen days of age, respectively. The thyroid lobes were found to be but loosely attached to the trachea, so that they were removed intact. The recurrent laryngeal nerves are not in contact with the thyroid, so there is no danger from this source in the pig. The operated pigs when returned to the mother, began to nurse immediately and in no case did they miss a feed. The young pig takes the ether quite readily, only a few inhalations being required to produce anesthesia. The wounds healed by primary intention in all cases and the sutures were removed on the seventh day.

Results. During the first two months no changes in physical condition could be observed. The operated and control pigs were of equal size and weight.

The first pig to show any change was the operated white sow. She developed many circumscribed, inflamed, swollen areas about the size of a dime, along the ventral surface of the abdomen, which were very resistant to treatment. The only other pig to show such lesions was the operated white boar. No specific cause for this condition could be determined, and in pigs, skin lesions of this kind are unknown to the writer. Scrapings from the diseased areas were negative when examined under the microscope. Owing to the slop feed which the mother received and the nature of the animals, the cement floor of the pen in which these animals were kept was wet most of the time, but plenty of clean dry straw was provided, and in general the pen was much cleaner and dryer than the average pig pen. This skin disease could hardly be attributed to the pen because only two of the pigs showed symptoms (both operated pigs) and such lesions are not common in pigs which are quartered in much poorer pens.

An outbreak of necrobacillosis existed among the young pigs of the university herd, but in these the lesions were confined to the mouth and internal organs. These sores on the operated animals did not become necrotic, nor could the necrosis bacillus be demonstrated. After several weeks the lesions disappeared, the affected animals being given plenty of sunshine and antiseptic, astringent baths.

The next indication of a lowered resistance was manifested by the operated black and white boar. In June he was noticed to be lame and upon examination, one of the digits of the left fore limb was found to be swollen and painful. No infection developed, the injury was probably a bruising of the tissues, which was very slow to heal. The animal remained lame for over six weeks. This condition is rarely seen in the pig and when bruising does occur the lameness is of short duration unless infection develops.

During the early summer when about three months old, all of the pigs showed symptoms of parasitism, especially lung worms, with much coughing. No treatment was given but this invasion interfered with the growth and thrift of all the pigs. The symptoms were more marked in the operated pigs and although the rate of gain was about equal in the two groups, the operated pigs made a much slower recovery than the normal. The operated white boar seemed to suffer the most, and became the smallest pig in the litter. His hair coat was rough, and the bristles came out. He became almost entirely bald over his body and the skin very red and congested. He developed a "pot-belly" and a myxedematous appearance. He was not expected to live.

In the fall when the cold weather set in, the pigs began to improve and gain in weight more rapidly and none of them showed any signs of parasitism. Even the white boar which was not expected to live showed a very marked improvement, although a little undersized. The pot-belly disappeared, the hair coat improved and other than being a little smaller he could not be differentiated from the others. The parasites did not interfere with the growth and development of the testicles in the male pigs, and it became necessary to castrate them. They were castrated on October 13, 1915, with the exception of the black and white thyroidectomized boar which was left entire, because it was intended to experiment with his fertility. No differences were noted in the size and appearance of the testicles of the operated and check pigs. The testicles represented 0.76 per cent and 0.56 per cent of the body weight in the check pigs and 0.77 per cent of the body weight of the operated pig.

The body temperatures of all the pigs were taken at frequent intervals, but they showed no variations from the normal.

During the winter the operated pigs grew well but did not gain in weight as rapidly as the check pigs. The average weight curve is shown in the following chart. It will be noticed that during the first few weeks the weights of both groups were equal; from the fourth to the ninth week there was quite a marked difference in the rate of growth in the two groups, but after the ninth week the rate of growth in the operated pigs was equal to that of the checks. This equal rate continued until the twenty-seventh week, when the rate of growth increased in the checks, but remained about the same in the operated pigs. At forty weeks, which is about the time of market maturity in this animal, there was an average difference of 30 pounds between the checks and operated pigs.

Sexually, all of the males were very active, but owing to the fact they were grade stock there was no opportunity to try out the reproductive powers of the thyroidectomized boar on mature breeding sows. Early in November the two sows (operated and check) came in heat. They were bred to the operated boar, and conceived, as dioestrum did not recur. Post-mortem on the two sows was held shortly before the time of parturition. The operated sow presented one *mummified fetus* in the uterus, and the check sow presented one *fully formed fetus*, which appeared normal and probably would have been born in a short time. This fact is significant, as normal sows bred to normal boars usually have five or six pigs at the first litter, and older sows may have as many as ten to fifteen. That the operated boar retained his sexual activity was demonstrated by the fact that whenever any of the sows in his building were in heat he made violent efforts to reach them and several times did get over the high partition of his pen.

Post-mortem findings.[1] On February 4, 1916, the operated white sow was reported sick by the feeder, and when seen was found to be in a dying condition. The feeder reported that for a couple of days she had not been eating very well. Post-mortem revealed pneumonia and pleurisy involving both lungs and the parietal and visceral pleura of both sides. The digestive organs showed a gastritis. Abdominal and thoracic lymph glands were congested. All other organs were normal, except the uterus; here there was considerable exudate and a mummified fetus was found. The head of the fetus was fairly well developed,

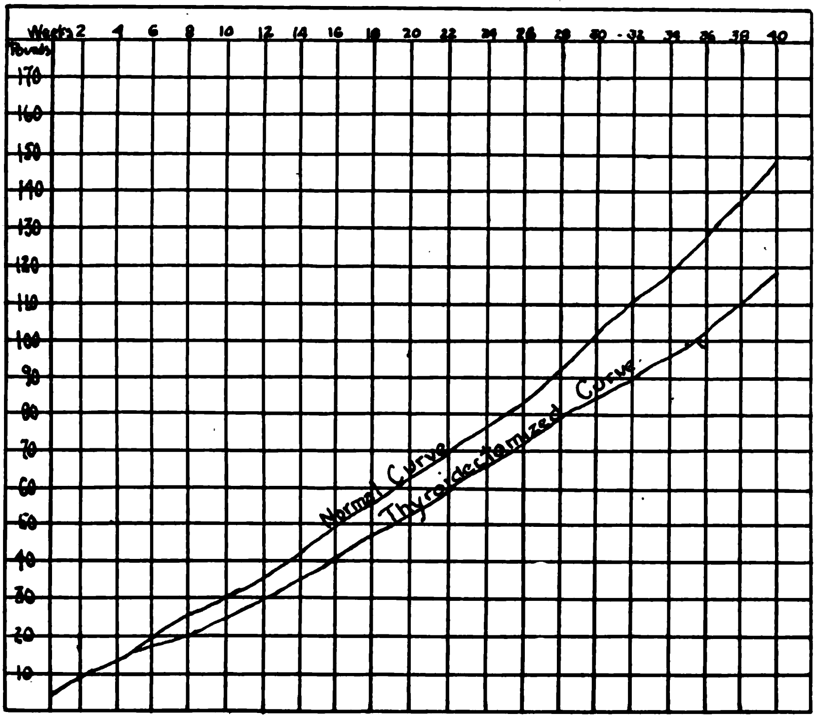

Fig. 1. The growth curves of three normal and three thyroidectomized pigs

the body small in diameter, but very long, and the legs were not developed to any appreciable extent. The region of the larynx and trachea of this operated animal was carefully examined and numerous gland-like structures varying in size from a small seed to a large bean were found and retained for microscopic study.

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[1] Dr. Kernkamp of the serum laboratories coöperated in the post-mortem examinations.

The pigs were carefully watched and none of the remaining animals showed any signs of sickness, but on February 14, 1916, two more were found dead. The pigs had been seen last by myself the day before and at that time they all appeared normal. The dead pigs were the operated white male and the check, black and white sow. The check sow was posted first, and the only lesions found were in the lungs. These organs showed lesions of pneumonia involving only the distal parts of the cardiac lobes. The uterus was normal and contained one normal fetus. The operated white male showed very marked lesions in many organs. There was extensive pueumonia and pleurisy. Digestive tract showed a congested intestine. The spleen was hemorrhagic and the kidneys showed a few petechiae. The lymph glands of the entire body were swollen and congested. All gland-like structures in the cervical region were removed and sectioned.

Although the lung lesions in the thyroidectomized animal were the predominating post-mortem findings, and the primary cause of death was very likely a pneumonia, the case resembled hog cholera somewhat. Therefore, when the remaining operated pig, the black and white boar, showed a slight temperature and was off feed a few days later, he was given 60 cc. of hog cholera serum. A blood inoculation would have been made from this animal to determine if he really had cholera, but no known susceptible pigs could be obtained at that time. The boar's condition remained about the same for a few days, then he grew worse, and died on February 20, 1916. Post-mortem examination revealed pneumonia and pleurisy involving both lungs and pleura, gastritis with blood stained stomach contents, lymph glands of thorax congested and swollen; kidneys showed a few petechiae, but all other organs normal. All glands from the cervical region were removed and sectioned as in the case of the other operated pigs.

The remaining check pigs did not become sick, the appetite remained good and they did not show any rise in body temperature.

All of the thyroidectomized pigs died, the female with the mummified fetus first, and the boar last. They all showed extensive lesions with those of pneumonia and pleurisy predominating and constant. Only one check pig died. Death in all these animals was, in the opinion of

Dr. Kernkamp and myself, caused primarily by pneumonia, which is rather common in pigs during the winter months. During cold weather the pigs will pile up on one another. Those on the bottom become very warm and later when they come in contact with the cold air, chill, and if their resistance is low are quite apt to develop pneumonia.

While some of these pigs showed slight lesions of cholera, they were not typical. Further, these pigs had been given serum when young and very likely became actively immune. If they were not, they would certainly have shown symptoms in the fall shortly after being placed in the shed occupied by virus pigs. The boar was given the serum when he first showed signs of sickness. Serum is sometimes effective as a curative agent for cholera, especially when administered early and in large doses.

At the time of the post-mortem examination all glands in the region of the larynx and trachea were removed and sectioned. These structures varied in size from a small seed up to that of a large bean or hickory nut. Most of the small glands proved to be lymph glands, but in each operated pig several glands were found which proved to be accessory thyroids. The glands were for the most part located at or near the thoracic inlet along the trachea. The largest accessory thyroid was found in the case of the black and white boar. The gland was 4 cm. long and 2.5 cm. wide and 0.5 cm. thick, located along the ventral surface of the trachea at the thoracic inlet. The alveoli in the accessory thyroids were about the size of those found in the thyroid of the week old pig.

Discussion of the results. Definite conclusions cannot be drawn from such a small number of animals, but owing to the fact that the variations from the normal in the three operated pigs were quite constant, we believe the following comments are warranted.

Our results are not in accord with those obtained by Horsley, Munk and Moussu, but are in general the same as those reported by Eiselberg.

It is difficult to account for the results obtained in Moussu's one case. One of our animals (the white male) when about three months of age began to show symptoms quite similar to those described by Moussu, and if the animal had died at this time we would have believed that this pig was developing into a cretin, but died before the syndrome was complete. This pig, however, soon began to improve and when fall came, looked and acted similar to the other pigs, though slightly undersized.

Moussu does not describe the method of feeding and quartering his animal, but it is well known that pigs and especially young pigs, if not fed properly and quartered in sanitary pens are quite likely to develop a pot bellied appearance, an unthrifty hair coat and general cachexia. Such a condition is usually the result of an invasion of parasites.

Another possible explanation of Moussu's results is, that the particular animal upon which he operated had a very small amount of accessory thyroid tissue. (In posting pigs I have sometimes found the large accessory thyroid, usually located at the thoracic inlet, to be absent.)

Horsley's pigs developed a condition of general cachexia and died after a long period of this depression. True cretinism would very likely have started to appear when the animals were much younger. Death in these cases was very likely the result of a lowered body resistance.

Although the details of Eiselberg's work are not available, our results are, in general, in accord with those obtained by him. Our operated pigs were somewhat undersized. This change was more marked during the last two months of their lives, and would have very likely increased if the pigs had lived longer. It is probable that this decreased rate of growth was the result of the lowered body resistance, or may have been due to disturbances in metabolism as a result of the diminished quantity of thyroid tissue.

All of the thyroidectomized pigs showed a marked lowered body resistance at some time during their lives. This fact is demonstrated by the skin disturbances, the parasitic invasion and finally the deaths from pneumonia. On post-mortem there were extensive tissue changes in the operated pigs which died from pneumonia, but only slight lung lesions existed in the one check pig which died from this disease.

Before the fatal pneumonia, a stranger had difficulty in distinguishing the operated from the normal pigs. Other than the slight differences in weight, there were no distinguishing clinical features.

The apparent effect upon the reproductive powers is interesting, but further work along this line is needed before any statements can be made.

SUMMARY

1. Extirpation of the main thyroid gland in young pigs does not induce cretinism, at least within a period of nearly one year. There is a slight retardation in growth, but the operated pigs act similar to the controls, except that they show a marked lowered resistance to infection.

2. Post-mortem examination of the thyroidectomized pigs revealed a marked hypertrophy of the accessory thyroid tissue.
3. The main thyroid gland plays an important part in the normal body resistance to infection and when this structure is removed the accessory thyroids cannot completely compensate.
4. In pigs a degree of hypothyroidism, not sufficient to lead to marked changes in physical appearance of the animal, lowers the resistance to infection to quite a degree, and impairs the functions of reproduction.

I wish to express my appreciation to Dr. Carlson for his valuable criticisms, and to Drs. E. P. Lyon, F. H. Scott and C. M. Jackson for their valuable suggestions.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

TWENTY-NINTH ANNUAL MEETING

New York City, December 27, 28, 29, 30, 1916

IN MEMORIAM

Isaac Ott, A.M., M.D., by Albert P. Brubaker.
Joseph Hoeing Kastle, Ph.D., by A. S. Loevenhart.
Allen M. Cleghorn, M.D., C.M., by Shepherd Ivory Franz.
Thomas Gregor Brodie, M.D., F.R.S., by A. B. Macallum.

The diastatic activity of the blood in diabetes. VICTOR C. MYERS and JOHN A. KILLIAN.

Although it has been recognized since the time of Magendie that blood possesses active diastatic properties, no studies of a conclusive nature appear to have been published relative to the activity of the blood diastase in human subjects suffering from diabetes. This has probably been due to the lack of a sufficiently delicate and accurate method.

It has been found that the procedure introduced by Lewis and Benedict[1] for the estimation of the sugar of the blood, may be excellently utilized in the estimation of its diastatic activity. Two 2 cc. samples of oxalated blood are taken, one being employed as a control. The control tube is made up to 10 cc. with distilled water and the tube to be employed for the test to 9 cc. Both tubes are now placed in a water bath at 40°C. As soon as the contents of the tubes have been brought to this temperature, 1 cc. of 1 per cent soluble starch is added to the second tube, the tube mixed, and incubation then carried out for exactly fifteen minutes at 40°C.

After the incubation has been completed, about 1 gram of dry picric acid is at once added to each tube and the mixtures stirred. When the proteins are precipitated, the tubes are centrifuged and the supernatant fluid filtered. The sugar in 3 cc. portions of the filtrates is now estimated according to the technique described by Myers and Bailey.[2] Correction is made for the sugar originally present in the blood (with the aid of the control) and for the slight reducing action of the soluble starch. The results have been recorded in terms of the percentage of the soluble starch (10 mgm.) transformed to reducing sugars (calculated as glucose) by the 2 cc. of blood employed.

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[1] Lewis and Benedict: Journn. Biol. Chem., 1915, xx, 61.
[2] Myers and Bailey: Journ. Biol. Chem., 1916, xxiv, 147.

It is believed that 10 mgm. of starch furnish a sufficient substrate for all ordinary conditions with human blood (except possibly in very severe diabetes), since practically identical results have been obtained with amounts of starch up to 40 mgm. Glycogen offers no advantage over soluble starch, since the results are essentially identical. The possible error of glycolysis would appear to be a negligible one during a fifteen minute period of incubation.

The diastatic activity of the blood, with this method, has been found to vary from 15 to 25 in a variety of miscellaneous conditions in the human subject. In cases of advanced nephritis with nitrogen retention and hyperglycemia, figures from 30 to 40 have been observed, while in eleven cases of diabetes the values ranged from 40 to 74. These results would suggest that the increased diastatic activity in both nephritis and diabetes (as shown by the analyses of the blood) may be the important factor in the production of the hyperglycemia in these conditions.

Observations concerning fat feeding. F. M. ALLEN.

The observed effects of fat feeding are classifiable in four divisions.

1. Effect on acidosis. It is well known that the ordinary laboratory animals show no ketonuria on fasting. There is no distinction between carnivora and herbivora in this respect. The experiments confirm the statement of Neumann and others, that young puppies show fasting ketonuria. Their weakness is the main difficulty for experimental uses. Adult dogs by partial pancreatectomy become subject to acidosis under three conditions:

A. Acidosis on feeding. Suitable fat diet gives rise to acidosis in severely diabetic dogs possessing sufficient power of fat digestion. Breakdown of digestion is the principal difficulty to be avoided. With successful management, the acidosis proceeds to typical diabetic coma. The fat ration employed has generally been 100 to 250 grams of suet daily. As in human diabetes, protein and carbohydrate fail to stop this acidosis, so that dogs go into coma on full mixed diet. The carbon dioxide capacity of the blood plasma falls only in the more severe later stage, but in coma approximates that of human patients. Doses of alkali easily maintain the blood alkalinity but do not perceptibly lengthen life. The excretion of acetone bodies is generally much less than in human patients, but the concentration in the blood is fully comparable.

B. Acidosis on fasting. When a dog with potentially severe diabetes is kept free from glycosuria and gradually fattened by means of a low protein, high fat diet, glycosuria ultimately develops and a condition can be attained in which the animal develops fatal acidosis on fasting, reproducing the condition and the symptoms of certain human patients.

C. Acidosis without glycosuria. Partial pancreatectomy creates susceptibility to acidosis even when glycosuria is entirely prevented by diet. Such animals on suitable fat diets develop ketonuria far more readily than normal dogs.

2. Effect on glycosuria. Fat feeding alone never gives rise to glycosuria, but in predisposed animals and patients free from glycosuria on a given diet, the addition of fat results in glycosuria.

3. Toxicity in normal animals. Young puppies are easily killed by excessive fat diet, especially by feeding pure fat after fasting for a few days has produced ketonuria. Adult dogs can be made to develop both acetonuria and intoxication if the fat feeding is pushed long enough. There is no parallelism between acetone bodies and intoxication in these animals. The symptoms are loss of hair, eczema, loss of weight, and very pronounced terminal ataxia and generally convulsions.

4. Lipemia. Partial pancreatectomy occasions only a slight lipemic tendency in absence of glycosuria, and glycosuria produced by phloridzin is known to be accompanied by only slight lipemia, but when the partially depancreatized animals are allowed to develop severe glycosuria, they become susceptible to true diabetic lipemia on fat feeding. As high as 10 per cent fat has been found in their plasma. The finer characteristics and the reasons for variations between individual animals are under study.

The experiments indicate that acidosis is not due solely to lack of carbohydrate, but that the pancreatic function is somehow concerned, possibly only indirectly through the liver or otherwise. A wide range of experimental material is afforded for researches not limited to diabetes, dealing with endocrine functions, enzymes such as lipases, lipoid metabolism from the standpoint of both chemistry and morphology, etc.

The cause of the increased heat production following pancreatectomy in the dog. John R. Murlin.

Three experiments were reported in which the heat production by the indirect method was determined before and after pancreatectomy; and, immediately following each respiration experiment, blood was drawn and examined for blood fat. In one experiment, also, the CO_2-carrying power of the whole blood was determined by the Haldane method. Thus it was possible to compare the heat production with the intensity of the protein metabolism (N output in urine) with the concentration of blood fat and with the actual acidosis.

Two of the dogs were fed both before and after pancreatectomy, one fasted throughout. In the fed dogs the heat production rose on the second day to 31 and 42 per cent, respectively, above the normal metabolism. In the fasting dog it rose to only 7 per cent above the normal. In the last-named dog the increased protein metabolism alone was sufficient to more than account for the heat production; but in the dogs which were fed it was not sufficient.

When fat is injected directly into the circulation[1] or when fat alone is fed[2] the increased heat production due to the dynamic action of fat runs parallel to the concentration of blood fat. But after pancreatectomy it does not. In none of the three cases did the maximum heat production coincide with the maximum concentration of blood fat. Since the carbon dioxid carrying power of the blood rose after pancreatectomy in the only case studied for this factor, the increased heat production can not be said to depend upon the acidosis. Furthermore, the exact course of the heat production, which was perfectly parallel for the two fed animals, could not be accounted for by any combination of the protein and fat factors. The subject is being investigated further.

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[1] Murlin and Riche: Proc. Soc. of Exper. Biol. and Med., 1915, xiii, 7.

[2] Murlin and Lusk: Journ. Biol. Chem., 1915, xxii, 15.

The use of cotton seed as food. THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

Both the *globulin* and the *total alkali-soluble protein* isolated from cotton seed as well as cotton seed *meal* and *flour* respectively are adequate sources of protein in the ration of growing rats. Small supplements of the meal or flour make the proteins of less efficient feeds such as "corn gluten," distillers' grains, etc., suitable for growth.

Cotton seed *meal* and *flour* contain "water-soluble" and possibly some of the "fat-soluble" vitamines.

Feeding with untreated cotton seed *kernels* leads to nutritive failure. They apparently contain something which is either toxic in itself, or renders the food so unpalatable that rats refuse to eat it. This results in so-called cotton seed injury. When the *kernels* are thoroughly extracted with ether or suitably steamed, rations containing them can be used with success. Food containing small quantities of the *ether extract* becomes unsuitable for nutrition.

Both the crude cotton seed *oil* prepared from heated kernels and that pressed from cold kernels can be fed without detriment. The cotton seed *residue* from the *cold pressed* oil is no better than the untreated kernels for nutrition.

Some of these facts corroborate observations by Richardson and Green and by Withers and Carruth.

The influence of certain conditions on the rate at which epinephrin is liberated from the adrenals into the blood. G. N. STEWART and J. M. ROGOFF.

1. Using rabbit intestine and uterus segment method for assaying epinephrin in adrenal vein blood, we have been unable to demonstrate any decided increase in the rate of liberation by stimulation of sensory nerves or asphyxia. This agrees with our previous results[1] by another method (collection of adrenal blood in a cava pocket and determination of its effect on blood pressure and pupil of the same animal when the pocket is released).

2. The concentration of epinephrin in the blood of the adrenal veins tends in general to increase as the rate of flow through the adrenals diminishes, the quantity liberated being proportional to the time rather than to blood flow.

3. For this, and possibly for other reasons the concentration of epi-

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[1] Stewart and Rogoff: Journ. Pharm. and Exper. Therap., 1916, viii, 479.

nephrin in adrenal blood tends to be greater in later than in earlier samples.

4. It follows that a change in the rate of liberation of epinephrin can not be deduced from a change in the concentration (as determined by segments or strips) unless the rate of flow of the adrenal blood has been measured.

5. Cava blood withdrawn by a catheter whose orifice is central to the adrenal veins does not in general give reactions with rabbit intestine and uterus segments which would permit the detection of epinephrin, even when the concentration in the adrenal vein blood is proved to be capable of giving marked reactions. The reason is the dilution of the adrenal vein contribution.

6. Even when serum is used instead of blood the results (for catheter specimens) have been negative. Yet when adrenalin is added to blood the separated serum contains practically all the adrenalin (by colorimetric, rabbit segment and blood pressure tests on the pithed cat).

7. Our attempts by the catheter method to demonstrate an increase in the rate of liberation of epinephrin by sensory stimulation, asphyxia and emotional disturbance have not been successful.

8. When the blood flow in the cava is sufficiently slowed the concentration of epinephrin in catheter specimens may be detectable with rabbit segments, although falling below the threshold of detection in earlier specimens when the cava flow was larger. This would not prove that the rate of liberation of epinephrin had been increased by any procedure concomitant with the slowing of the blood flow. For if the rate of liberation remained the same, the concentration in the catheter blood would necessarily increase as the flow in the cava lessened.

The blood lipoids in nephritis. W. R. Bloor.

Lipemia has occasionally been reported in nephritis and because of its importance in the study of fat metabolism an examination of the blood lipoids in a series of cases of severe nephritis was undertaken. The results showed mainly marked increases in the total fatty acids (due to fat) in the blood plasma and of lecithin in the corpuscles, with less marked and less frequent increases of fat and of cholesterol in the corpuscles. These abnormalities are essentially those found in the blood in alimentary lipemia and their presence in nephritis is believed to be due to a retarded fat assimilation which is regarded as one manifestation of a general phenomenon produced by the acidosis present in this condition.

Concerning the lipoids of the blood in renal conditions, with special reference to the cholesterol content. Albert A. Epstein and Marcus A. Rothschild.

In the interpretation of phenomena associated with renal conditions, the changes found in the blood and urine are ordinarily attributed to the pathological process and disordered function of the kidneys. By a comprehensive study of the chemistry of the blood effusions and of the urine in renal and other conditions, the conclusion has been reached that a type of renal affection exists, commonly known as chronic nephrosis, in which the pathological and chemical basis is that of a nutritional disorder. One of the authors (Epstein) has on several previous occasions pointed out the fact that amongst the cases of chronic parenchymatous nephritis there is a group which is essentially different from all other renal affections, and is fundamentally a constitutional disturbance.

This conclusion is based on the profound changes, particularly in the protein content of the blood serum, encountered in this type of nephritis, and also from the character of the chemical composition of the effusions as compared to that observed in other conditions.

By means of the newly developed methods for the examination of fats and lipoids in the blood, it has been possible for us to examine nearly one hundred cases of renal disease and allied conditions. The results may be briefly summarized as follows: In chronic nephrosis of the type referred to, particularly in an edematous stage, the lipoids of the blood are exceedingly high; the cholesterol content, to which special attention has been given in this study, has been observed as high as 1.226 grams per 100 cc. In other renal conditions, including surgical cases, no such increase has been encountered. Occasionally a moderate rise has been found, but as a rule this was associated with some other superimposed condition.

In uremic cases, particularly those showing high nitrogen retention in the blood, the lipoid content is very much diminished. The cholesterol in such cases may fall as low as 0.080 gram per 100 cc. Several effusion fluids from cases of chronic nephrosis have been examined and only traces of lipoid have been found in them. The urines of such cases, when rich in protein material, show the presence of small amounts of lipoid.

The origin of the increase of the lipoid content of the blood in the parenchymatous form of nephritis is not quite clear. Undoubtedly a large portion is due to ingested lipoid material. A certain part may be ascribed to mobilization of body fat, particularly as such cases when recuperating show extreme emaciation. This high-grade form of malnutrition is undoubtedly associated with considerable tissue degeneration, so that much of the lipoid in the blood must be attributed to that source. At any rate, the finding evidently represents a condition of fat non-utilization comparable to that found in diabetes and other nutritional disorders. This is evidenced also by the fact that when a diet consisting of large amounts of protein, a small amount of carbohydrate, and practically no fat, is administered for a long period of time, the excessive lipemia and lipoidemia gradually subside.

Observations on acid base equilibrium in the body. W. McKim Marriott and John Howland.

Known amounts of hydrochloric acid, of acid sodium phosphate and of sodium phosphate of blood reaction were administered to normal adults. Determinations on the urine showed that hydrochloric acid administration led to an increase in titrable acid (A) and a proportionate increase in ammonia excretion, the ratio A/NH_3 remaining constant. Acid sodium phosphate increased the titrable acid excretion but had no effect on ammonia excretion. The ratio, A/NH_3 was greatly increased. Administration of phosphate mixtures of blood reaction ($p_H = 7.4$) led to a slight increase in the titrable acid and to a distinct diminution in ammonia excretion. The A/NH_3 ratio was considerably above normal limits.

These findings may be taken to explain, in part, at least, the low excretion of ammonia in the acidosis of nephritis, it having been previously demonstrated that this condition is accompanied by a retention of acid phosphate.

The movements of the artery within the compression chamber during indirect estimations of the blood pressure. JOSEPH ERLANGER.

This report is based mainly upon records (made with the aid of a compression chamber applied to the dog's femoral artery) reproducing the movements of the walls of the artery within the compression chamber. The records made during steady decompression show that the pulse does not penetrate the full length of the compressed artery when it first enters it; rather the penetration depth increases at the rate of 1 cm. per pressure decrement of 2 mm. Hg. Once blood has entered the artery there is not time enough between pulses for the artery to completely empty itself. This residue of blood increases rapidly at first until a thickness of about 0.08 to 0.24 mm. is attained, then more slowly if at all, and again more rapidly and at an accelerating rate before the fourth sound-phase begins. This changes to a retarding rate of increase with the first of the fourth sound-phase pulses, because the artery at this time fails to collapse during diastole beyond its full but undistended size. During the whole of the first-to-fourth sound-phase the pulse, especially in the lowermost parts of compressed artery, shows a pre-anacrotic negative wave. This wave, as demonstrated by other methods, develops when a pulse passes through a localized compression constriction of a tube. It disappears when the tube is no longer constricted below its full but undistended size. The first sound-phase begins shortly after the pulse penetrates beyond the lowermost parts of the compressed artery; the fourth sound phase, at the instant during diastole the artery fails to pass below its stretched size. The behavior of the anacrotic limb of the pulse, as well as certain other phenomena, furnishes evidence in support of the water hammer theory of sound production. The anacrotic limb varies in such a way as to account for the sounds of the first, third and fourth phases. The elevation of the crest of the pulses increases rapidly at first, then more slowly, and again very rapidly as the fourth sound-phase is approached. The last rapid increase is mainly the result of the damming back of blood. The amplitude of oscillation at any point on the artery is given by the difference between the systolic and diastolic levels. The oscillation curves have the usual form, but the sudden diminution in amplitude usually seen at diastolic compression is exhibited best, sometimes only, by the middle region of the compressed artery.

The veno-pressor mechanism. YANDELL HENDERSON.

By the veno-pressor mechanism I mean a third factor in the circulation which regulates the venous return to the right heart—the heart and the control over the arterioles by the vaso-motor nervous system being the other two. The following experiments throw some light on its nature. When the intestines are traumatized, either mechanically, or with mustard oil, the efferent vessels (mesenteric veins) contract and dam the blood back in the tissues, thus producing congestion and decreasing the return of the blood to the heart. This occurs equally well after section of the splanchnics. It is the essential feature of circulatory failure in experimental shock. The phenomenon is essentially the first stage of inflammation and is possibly related to the observations of Ninian Bruce on this matter. The same mechanism is affected by forced breathing in a normal man, as can be demonstrated when venous pressure is determined with the subject lying head down at an angle of 30°. The measurement of venous pressure in this position renders the estimation of this important function feasible to an extent otherwise unattainable, especially when as in sickness the venous return to the heart is diminished.

The effect of pneumothorax in the dog and cat. H. C. JACKSON and CHAS. J. IMPERATORI.

By pneumothorax in the dog and cat we mean a *double pneumothorax.* Single pneumothorax with the existing apparatus and our present methods is impossible to obtain. It would seem that some observers are of the opinion that there is a natural or anatomical opening between the two pleural cavities. Repeated dissections done on dogs but mostly on cats seem to show that there is no natural or anatomical communication. Test animals were bled to death and then immediately fixed in formalin solution.

Methods of inducing pneumothorax were, (a) by puncture of the chest wall, or (b) by puncture of the lung from within a bronchus, thus gaining entrance to the pleural cavity. Practically all the work was done by puncture through a bronchus.

Immediately following puncture the animal shows signs of embarrassed respiration and is dead in from two to four minutes from what might be termed a very rapid asphyxia.

Animals that were punctured from within the bronchus died more rapidly than those in which the chest wall was punctured. The normal negative pressure in both pleural cavities after puncture immediately becomes positive. There does not seem to be any appreciable interval of time between the change from negative to positive pressure on the side *not* punctured. Autopsy findings were collapse of *both* lungs regardless of which side was punctured.

Post mortem examinations all showed a collapsed condition of both lungs and a rupture of the pleural septum just behind the sternum, either a tearing of it or in the majority of instances a sort of peppering of this septum with small holes.

Simultaneous collapse of both lungs by the careful use of fluids, colored fluid being used on one side, resulted in the colored fluid remaining in its side and in death from asphyxia. But if the animal made violent efforts at respiration, a rupture of the septum invariably resulted. It might be of interest to note that those animals where the lung was punctured by the bronchoscope and air admitted to the pleural cavities, and who were rapidly becoming asphyxiated, could be resuscitated by puncturing the chest wall. By closing this opening in the chest wall they could again be asphyxiated; this procedure could be repeated many times, that is, alternately asphyxiating and resuscitating the animal.

Pneumothorax and the subsequent collapse produce, as it were, a clamping off of the pulmonary vessels and a rapid asphyxiation.

Effects of physical training on pulse and blood pressures during activity and during rest. PERCY M. DAWSON.

The subject of these studies is the writer. The periods of observation were two to three months long, one period being in each of the years '14, '15 and '16. During these periods blood pressure and pulse rate determinations were made from four to seven times a day. Only four series of these observations are included in this paper, namely, those made on rising, at noon, in the late afternoon, before retiring. The auscultatory method was used in blood pressure determinations, four to ten readings being taken on each occasion and the average being then computed. In '14 the subject frequently ran three miles; in '15 he performed gymnastic exercises; in '16 played tennis. It was therefore possible to study the effect of physical exercise on the resting pulse and blood pressure and also upon the cardio-vascular reaction to standard physical tests (cycle ergometer). The following are the more important results.

Resting pulse rate is decreased especially in '14 and '16, and the extent of the diurnal variation is decreased.

Resting blood pressure observations are most complete in '16. They show that both pressures are increased on rising and in the evening, both fall at noon but in the afternoon the rise in systolic pressure is accompanied by a fall in the diastolic. The diurnal variations are decreased but suffer no qualitative change.

Resting pulse pressure is increased in all cases.

Resting pp × pr is affected neither conspicuously nor constantly.

The effects of several weeks of systematic exercise upon the blood pressure reaction to physical tests (cycle riding) are these: (1) the systolic pressure rises much higher (cf. 170 and 206); (2) the diastolic after a short rise falls to or below normal before the completion of the test; (3) the pulse pressure is much greater (cf. 62 and 114); (4) the pp × pr is greatly increased (cf. 6820 and 12540); (5) the after effects (negative phase and fall in diastolic pressure) are considerably reduced.

If the untrained subject performs two cycle tests with a day of rest between them, the first ride is seen to modify very greatly the cardiovascular reaction to the second ride. This has been studied only in the period immediately following the exercise. We then see a higher systolic, a lower diastolic, an increased pulse pressure (cf. 46 and 60) an increased pp × pr (cf. 5980 and 7800).

During the period of observation the subject caught "cold" twice, once before and once after the period of training. To the subject the two infections seemed to be identical in character, duration and severity. The only objective sign on both occasions was the increase in pulse rate. The maximum in the first case was 86 and in the second 69.

Some electrical phenomena of animal tissues. ROBERT GESELL.

Renal electrical variations were studied during sodium sulphate and "saline" diuresis. Though the details of the deflection varied the usual result was in each case increased negativity running roughly parallel with the secretion.

Certain exceptions—such as reversal of the electrical variation, the occurrence of electrical variations though no noticeable change in renal activity occurred, and the production of copious diuresis unaccompanied by marked electrical variations pointed out difficulties in the interpretation of secretion by means of the accompanying electrical changes. The problem temporarily resolved itself rather into the interpretation of the electrical changes than the interpretation of secretion by electrical changes.

Of the tissues studied, with the exception of the testicle, decreased blood supply produced a decreased negativity. In the case of the salivary glands, two results were obtained, following pilocarpin injection. If the salivary secretion was accompanied by increased negativity, decreased blood supply produced the usual decreased negativity. If the secretion was accompanied by a reversal of the usual electrical variation, decreased blood supply produced increased negativity.

The data from the use of adrenin, pilocarpin and direct stimulation of nerves as yet are not sufficient for reaching a generalization concerning the electrical effect of sympathetic and autonomic stimulation.

The electrical variation of the submaxillary gland of the dog produced by faradic stimulation of the chorda tympani showed four components which to a certain extent were under experimental control. The first component was produced by a short weak stimulation. The second component was added by a stronger stimulation and the third by a still stronger or longer stimulation. The fourth component seems to vary with the intensity of stimulation.

Obstruction of the salivary duct just prior to stimulation of the chorda tympani changes the contour of the deflection.

If all the components of the electrical variation produced by stimulation of the chorda tympani were present during secretion produced by the injection of pilocarpin they were fused into a smoother curve.

Obstruction of the salivary duct during secretion following injection of pilocarpin produced a marked deflection, so did release of the obstruction.

Injection of atropin after effective pilocarpin injection produced a sudden decrease of the original deflection with stoppage of secretion.

Stimulation of the chorda tympani after injection of atropin sufficient to stop or prevent a noticeable secretion still produced a small electrical variation. A method was devised for recording electrical variations on smoked paper.

Experiments on the relation of blood pressure to urine formation. A. N. Richards and O. H. Plant.

By a method previously described[1] rabbits' kidneys were perfused with hirudinized blood under conditions which not only permitted the regulation and measurement of blood flow through the organ, but also prevented alterations in blood flow as a result of changes in resistance in the renal circulation. Keeping blood flow constant, increase of blood pressure in the renal circulation by (1) partial obstruction of the renal vein; (2) stimulation of the splanchnic nerve; or (3) injection of adrenalin was found to increase urine elimination.

Evidence was presented which indicates that adrenalin causes constriction of the efferent vessel of the glomerulus and therefore, under the conditions of these experiments, causes increase in pressure in the glomerular capillaries. For the volume of the kidney, perfused at a constant rate, may show no change or may undergo increase after the injection of amounts of adrenalin which cause marked constriction of its vessels as shown by rise of pressure in the renal artery; whereas the forelimb or a loop of intestine, perfused under identical conditions, invariably decreased in volume after any dose of adrenalin which caused constriction of its vessels. This fact is taken to mean that a passive distension of the glomeruli results from constriction of the vasa efferentia which is great enough to prevent shrinkage of the kidney.

The fate of phenolsulfonephthalein when injected into the animal organism; factors other than the kidney influencing its retention. E. C. Kendall.

Phenolsulfonephthalein may be destroyed within the animal organism by being reduced. When once reduced, it is not possible to reoxidize the substance within the body to its original color. Blood fully oxygenated and reduced will not destroy phenolsulfonephthalein. Therefore, the destruction is caused by a reducing substance within the tissues and not within the blood. The oxygen of the blood is sufficient to fully meet the demands of the reducing action of the tissues, and will prevent a destruction of the dye. As high as 98 per cent may be recovered during a continuous injection of the substance provided glucose is the substance being metabolized. When amino acids and fats are being metabolized, the amount recovered may be much less. Fundamentally, the phthalein test measures the relation between the supply of oxygen of the blood and the reducing power of the tissues.

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[1] Journ. Pharm. and Exper. Therap., 1915, vii, 488.

Factors other than the kidney which influence its so-called retention are poor oxygen-carrying capacity of the blood, sluggish circulation, improper aeration by the lungs, and any other factor which produces asphyxia or a condition of acidosis.

The action of diuretics on the denervated kidney. WILLIAM C. QUINBY.

That the plexus of nerves going to the kidney has a marked vasomotor function is everywhere admitted. Besides this action, however, certain observers have claimed that some of these nerves were of specific secretory function. By others this has been denied. Certain investigators attempt to show that the vagus carries such secretory fibers to the kidney, while others attribute such to the splanchnics.

The difficulty in reaching a conclusion on the subject has been in the main due to two causes. In the first place, stimulation or section of vagus or splanchnic causes such marked vasomotor changes that any possible secretory effects are easily overshadowed; in the second place, the operation which attempts the division of the plexus of nerves at the renal pedicle has given inconclusive results because of the uncertainty that all the nerves were cut.

To meet this difficulty it was determined to study the function of a kidney which had been removed from the body and subsequently restored to its normal circulation by means of suture of the blood vessels. In this way it is possible to be sure that the kidney is freed from all nerve control, for a time at least.

The results of this portion of the work have been reported in the Journal of Experimental Medicine for April, 1916.

By transverse incision a dog's kidney was made accessible. After being surrounded by silk cloths, impregnated with liquid vaseline, the structures forming the pedicle of the organ were cut, and the kidney removed from the body. A suture of artery, vein and ureter was then made, by which the circulation was restored after an interruption of about one hour, At varying periods after this operation the function of the kidney was studied by. comparing it with that of the intact kidney.

The result obtained from this series of observations showed that the function of the reimplanted kidney was greater than normal during the days immediately following operation until about two weeks had passed, when it was found normal.

These observations, together with the fact that it has been repeatedly possible for a single reimplanted kidney to maintain normal existence of the dog after removal of the kidney on the other side, tend strongly to exclude the existence of any specific secretory nerve fibers.

In this present communication the action of diuretics on such a reimplanted kidney has been studied. As examples of the three types of diuretics, sodium chlorid in hypertonic solution, urea, and caffein or diuretin were used. It is possible that such diuretics as do not cause increase of general blood pressure may act by stimulation of secretory nerves if we suppose such to exist.

In order to register the action of the injected substances the kidney was placed in an oncometer of the type designed by Roy, and by it changes in the volume of the organ were recorded on the kymograph. Observations of flow from the cut ureter of this kidney were also made.

A series of observations was first recorded from normal animals, after which the same method was applied to those bearing a reimplanted kidney.

It was found that the type of reaction shown by the normal and reimplanted kidney is practically identical for the same diuretic.

Here again, then, as in the series of observations already reported, we are unable to find any evidence of abnormality on the part of the reimplanted kidney, its response to diuretics being the same as that of the intact organ. In other words, the reimplanted kidney shows no evidence of loss of any possible secretory nerve influence.

On the movements of the isolated ureter of the dog. GEORGE B. ROTH.

It was found that longitudinal segments and strips, as well as ring preparations of the kidney-, middle- and bladder-third of the isolated ureter of the dog could be made to beat rhythmically for many hours when placed in oxygenated Locke's solution which had a H-ion concentration of 8.3.

Two types of contractions were seen in most experiments, namely, large and small. These types were produced not only by segment preparations but also by the strip preparations in which the circular fibers were cut, so that both types are apparently due to the longitudinal fibers. The large type contractions consume about one and one-half to four seconds in contracting and from seven to seventeen seconds in relaxing. The small type may be more sluggish and sometimes require six to eight seconds for contraction; relaxation requiring twenty to thirty seconds. The small type contractions predominated in the kidney-third; while in the middle- and bladder-third the large type were most frequently found. Relaxation which was independent of contraction was seen in a few experiments in which kidney segments were used.

The rate of the contractions in the various parts of the ureter varies from 4 to 50 in ten minutes. In any segment the contractions are relatively infrequent at first and later become more frequent. The amplitude of the contractions also varied; as a rule segments from the middle- and bladder-third contracted more vigorously than those from the kidney-third.

Epinephrine and barium were shown to stimulate all parts of the ureter. This was shown by an increase in the tonus, rate and amplitude of the beating segments. After apocodeine the epinephrine effect is abolished or lessened, while the response to barium is practically normal, thus indicating that autonomic nerve endings are present throughout the ureter.

Apocodeine first stimulated then depressed ureteral activity. Cocaine and novocaine produced stimulation.

Monobasic acid sodium phosphate caused depression of ureteral movement, which could be counteracted by the addition of small amounts of sodium bicarbonate.

The stimulating action of the bromide-ion on smooth muscle. THEO. K. KRUSE.

Experiments were made on isolated strips of intestines of the cat and dog. Other types of smooth muscle were also studied such as the oesophagus, stomach, intestines of a frog and turtle and of the oviduct of a turtle. The usual suspension technic for smooth muscle was used. The chloride salts of a Ringer's solution were replaced by the corresponding bromide salts. The action of a chloride and a bromide Ringer's solution on smooth muscle was compared.

The results of the investigation may be summarized in the following manner:

1. The action of the bromide-ion is stimulative in nature. This stimulation falls on tone, amplitude and rate. The change in tonicity is the more constant reaction.
2. When two strips are set up at the same time in each solution, the one in the bromide solution usually responds first.
3. With no preëxisting rhythm in a chloride bath, rhythmicity is frequently induced by changing the strip to a bromide bath. If at this time, it should be changed back to the chloride bath, the rhythm may again be abolished.
4. A strip of low or decreased activity responds with greater tone changes than does a rhythmic strip. This seems to be associated with tissue oxidation.
5. Frogs are less responsive to these reactions than are the mammals.
6. Frequently a regularizing influence is observed during the bromide application.
7. An attempt has been made to associate these reactions, in part, to changes in tissue respiration.

The physiology of the chromatophores of fishes. II. Responses to alkaline earths and to certain neutral combinations of electrolytes. R. A. SPAETH.

In dilute sea-water the melanophores of *Fundulus* remain expanded for some hours. The individual salts of sea-water, however, with the single exception of NaCl, cause a contraction of the melanophores. The effects of various combinations of the salts of sea-water and the chlorides of Sr and Ba have been studied.

$CaCl_2$ is a less effective contracting stimulus than is KCl both in pure solutions and combined with NaCl. In comparing the effects of $CaCl_2$ and NaCl upon the KCl contraction it appears that $CaCl_2$, itself a contracting salt, increases the time of the KCl contraction more than does an equivalent amount of NaCl. The addition of a relatively small quantity of $CaCl_2$ to a contracting mixture of NaCl and KCl ($Na:K = 5:1$) prevents the normal contraction. Since the normal contraction is undoubtedly due to KCl, the $CaCl_2$ inhibits the action of the KCl. If the $CaCl_2$ is added after contraction has occurred, the melanophores promptly expand. Thus, in this case, the addition of a contracting salt actually produces an expansion of the melanophores. $CaCl_2$ inhibits the KCl effect only to an optimum concentration. The addition of further $CaCl_2$ produces a more and more rapid contraction. A comparison of the inhibitory action of $CaCl_2$, $MgCl_2$, and $MgSO_4$ shows that they are effective in the order named.

Comparative studies upon the effects of the chlorides of Ba, Sr, Ca and Mg have shown that as regards the speed of the initial contraction, the liminal concentration for the inhibition of the KCl contraction, the time required for complete recovery and the toxicity, the order is

$$Ba > Sr > Ca > Mg$$

The recovery from Ba is peculiar in initiating a pulsation of the melanophores. By means of the methods described in previous publications, all of the above results have been graphically represented and will appear shortly in a detailed publication.

The absorption of fat in partially and in completely depancreatized dogs. CHARLES W. McCLURE, BETH VINCENT and JOSEPH H. PRATT.

It was found that dogs with a subcutaneous pancreatic transplant secreting and discharging pancreatic juice externally absorbed no more fat than a dog in which the pancreatic remnant was undergoing rapid atrophy and sclerosis. Hence, the condition of the pancreatic tissue remaining in the body does not influence the amount of fat absorbed by the intestine.

The absorption of fat by the intestinal mucous membrane is always markedly disturbed when the pancreatic secretion is excluded from the intestine.

After the complete removal of all pancreatic tissue from an animal, the absorption of considerable amounts of fat can still take place.

Absorption experiments were made on three completely depancreatized dogs. The fat absorption ranged from 17.6 per cent to 45.6 per cent; the amount absorbed from 19.8 grams to 179.4 grams; the amount per kilo of body weight from 1.7 grams to 17.9 grams.

The visible structure of cell protoplasm and death changes. ROBERT CHAMBERS, JR.

1. This paper deals with results obtained on dissecting living cells under high powers of the microscope by means of fine glass needles held in Barber's mechanical pipette holder.

2. Protoplasm is a hydrophyllic colloid which, in cells such as early germ cells, egg cells and protozoa, usually exists in the sol state with a surface layer in the gel state. Adult somatic cells generally are gels in which one can not demonstrate a cell membrane possessing a consistency different from that of the cytoplasm within.

3. The microscopically visible granules in the cytoplasm of the egg of Arbacia may be classified into two types: (a) The microsomes, which are considerably less than 1 micron in diameter and constitute the most resistant parts of the cell maintaining themselves after complete disorganization of the cell, (b) the macrosomes, which range from 1 to 4 micra in diameter and are extremely sensitive to injury.

4. The external surface of the egg cell is a gel which passes gradually into the sol in the interior. The surface gel is very extensile and contractile and is readily regenerated on injury. Tearing of this surface, if unrepaired, results in the pouring out of the internal cytoplasm and dissolution.

5. A remarkable property of protoplasm is its ability to form a protective gel film not only on its external surface but also around an injured area which is in the process of disorganization. The disorganized mass thus insulated is eventually expelled from the cell.

6. A continuous but gradual application of mechanical injury can be sustained by a cell for some time without evidence of harm done. A short but rapid application produces instant local destruction, the spread of which may involve the entire cell.

7. Injury is accompanied by a swelling and an apparent increase in the acid reaction of the part involved.

8. Disorganization of the cytoplasm of the egg cells studied takes place in the following way: first, the megasomes swell and go into solution; and second, the liquid hyaline cytoplasm may flow out and disappear in the surrounding water or it may suddenly set, forming a rigid coagulated mass. The coagulation structure gradually coarsens with the production of a network or granular precipitate.

The effect of a-amino acids and peptones on the growth of cells in vitro.

CLARENCE A. NEYMANN and MONTROSE T. BURROWS.

The observations recorded were noted during a series of studies directed towards finding an artificial medium for cultivating cells *in vitro*. In the tissue culture the nutrient materials for the growing cells are derived from the cells disintegrating in the centre of the fragment of tissue. The culture reaction is none other than a transfer of materials from these cells in a less favorable environment to the growing cells on the periphery of the fragment which are in a more favorable environment. Previous evidence had given rise to the belief that the body cells are fluid-like structures. They are unable by themselves to break down complex substances and to build the products thus liberated into their own protoplasm. Growth in these cells is a reaction between themselves and a specifically organized mechanical and chemical environment. It is a differential surface tension reaction. There is a definite period for the growth of cells about a given fragment which has been placed in a medium of blood plasma. Transplanting fragments every three days, we can predict the number of transplants in which growth may be observed. This differs with different tissues.

We tried the effect on this reaction of the addition of carbohydrates, fats, peptones and a-amino acids to the medium. Fragments of heart muscle and skin of chick embryos and foetal chickens were the tissues used. They were planted in plasma, prepared from the blood of adult chicken. The addition of carbohydrates and fats had no effect of present interest. The addition of a 2.5 per cent isotonic solution of peptones prepared from the yolk of eggs had no effect. The addition of an isotonic solution of a mixture of a-amino acids, prepared by hydrolizing egg yolk proved toxic. Fearing this toxicity might be due to the method of preparation we tried the addition of isotonic solutions of the individual a-amino acids. These have also proved to be toxic when used in certain concentrations. The cells of young embryos are killed after a few days' growth in the first culture. The cells of older embryos and foetal chickens are more resistant but they are killed after a few transplants. Lower dilutions also inhibit the growth and eventually kill. At first, however, they show slight stimulation. This is a functional rather than a growth stimulation. Fragments of heart muscle were used. The stimulation is noted by the active contraction of the fragment.

Muscular Irritability. C. C. GUTHRIE.

The studies were made on skeletal muscle-nerve preparations of frog, and heart tissues of frog and terrapin. Possible technical errors were controlled.

Augmentation. Strong stimulation increases response of skeletal muscle to subsequent single stimuli. In magnitude, the increase may be hundreds of per cent. The period of increased response varies with strength and degree (rate, and strength of stimuli), and with the state of the preparation. The phenomenon is evoked only when certain relationships between state of tissue, and strength and rate of stimulation are realized. The effects are obtained when all stimuli are applied either to nerve or muscle, or alternately to the two, i.e., tetanizing muscle directly and then stimulating nerve with single stimuli, or *vice versa.* Other evidence that nerve terminations may contribute to the effect was presented.

Depression. Stimuli applied to heart during the absolute refractory period may cause an extension of the refractory period. If of suitable strength, and if such stimuli be repeated at short intervals, a further marked extension of the refractory period may be produced. If stimulation be discontinued for a short time and then a single stimulus applied, the muscle contracts. This phenomenon may be observed on isolated ventricular or auricular tissue and succeeds after atropin, so it seems to be independent of vagus action. Studies with natural impulses were included. High frequency current effects on skeletal muscle give evidence of an extension of refractory period. Interpolated single stimuli also depress, but quantitatively the effect is small.

Discussion. Underlying fundamental processes concerned in augmentation are, no doubt, of chemical and physico-chemical character. Such processes may result in increased irritability; increased contractility; slowing in muscular relaxation; participation of more contractile elements, due (a) to increased irritability, (b) facilitation of conductivity; facilitation of summation; a participation of two or more of the above factors.

A factor probably concerned in the differences observed in augmentation is the greater preponderance of depressing fatigue effects in certain instances. It seems reasonable to infer that augmentation of fundamental processes, although present, may be obscured by such fatigue influence, since our method of judging augmentation is the algebraic sum of two or more effects. Depressing influences more directly associated with stimuli also must be considered. But in general, it seems best to consider that the differences in the factors are more of degree than kind. Factors latent in certain conditions may become manifest under other conditions. Depression is probably best interpreted by giving major consideration to extension of refractory period. A tentative classification of the phenomena was suggested.

Studies in muscular power and fatigue. A. H. RYAN and J. H. AGNEW.

The work reported (on man) consists of (a) a new instrument for analyzing the tension changes occurring when a muscle is contracted either in shortening or isometrically; (b) the application of the results obtained to a critique of previous methods; (c) a new machine for measuring muscular power; (d) results.

(a) The instrument consists of a pair of steel springs arranged on a frame, in such a way that the muscular contraction produces an expansion of the springs which moves a lever over a row of brass contacts set in fiber, closing electric circuits. Each contact is accurately calibrated so that the tension curve can be determined.

(b) Tension multiplied by time has been taken as the index of muscular performance. When the tension-time curve is compared with the work curve in the Mosso method, the former is seen to undergo an irregular increase as the latter decreases. Minute analysis of the individual curves shows why this must necessarily occur as fatigue comes on. Such analysis also casts doubt upon the importance of devices for unloading the muscle during relaxation, also on the interpretation of results obtained with Mosso's ponomètre. The Treves and isometric spring methods were also studied. Mosso's method is considered to have essential faults in conception, while objections are raised to the other methods investigated.

(c) The new instrument is constructed from entirely different conceptions. Tension-time is taken as the index of muscular performance, but the muscular performance has been unified by an arrangement in which the weight decreases as the muscle shortens, in a manner corresponding to the decrease of contractile stress of the muscle (principle of Fick's cosine lever). The end-point is definite, and unpleasant sensations of fatigue are absent. Certain associated unconscious movements were also measured, for the purpose of throwing some light on the course of the voluntary effort under different conditions.

(d) Great normal variation in muscular power was seen. The performance was frequently greater in the evening than in the morning, and frequently very high after a loss of rest. Control subjects were used in studying the effect of barometric pressure, and it was found that their curves followed different courses; and that no relation could be established between barometric level, or direction, and muscular performance. The performance under caffein was much greater than the low or average level, but no greater than the high level of normal muscular performance. The greatest caffein effect was observed four to six hours after administration of the drug. Results obtained in the associated movements will be reported later.

A comparison of the amount of catalase in the muscles of active and inactive animals. W. E. Burge.

A comparison of the catalase content of the leg muscles and back muscles of active wild rabbits and inactive tame rabbits, of active gophers and inactive rats, of active ground hogs and inactive dogs, was made. A comparison was also made of the catalase content of the leg and breast muscles of active wild pigeons and inactive bantam chickens. After these animals had been washed free of blood by the use of large quantities of 0.9 per cent sodium chloride the muscles of which the catalase content was to be determined were removed and ground up in a hashing machine. One gram of the material was added to 45 cc. of hydrogen peroxide in a bottle, and as the oxygen gas was given off it was conducted through a rubber tube to an inverted burette previously filled with water. After the gas thus collected was reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the gram of muscle.

The average amount of oxygen liberated by 1 gram of the breast muscle of six pigeons was 91 cc., the average amount of six bantam chickens, 7 cc. By comparing these amounts of oxygen it will be seen that 1 gram of the breast muscle of the pigeon contains about thirteen times as much catalase as that of the breast muscle of the bantam chicken. The pigeons were able to fly practically indefinitely while the bantam chickens could scarcely fly at all. A comparison of the catalase content of the corresponding muscles of the other animals showed that the active muscles were richer in catalase than the inactive muscles, but there was not such a marked difference in any of the muscles compared, as between the breast muscles of the bantam chickens and the pigeons because there was not so much difference in the amount of work done.

A study of the total and preformed creatinine in various muscles of the cat. Ernest L. Scott and Adelaide Spohn.

Cats were used throughout the work. They were killed by decapitation. The muscles were then removed and cleaned of connective tissue and fat either at once or after they had been fatigued. The determinations were made by the methods described by Folin.

1. Do the several muscles contain specific amounts of creatine and creatinine? The muscles studied were the diaphragm, the extensor longus digitorum, the soleus and the bladder. The results obtained from a series of 23 animals indicate that each muscle contains a specific amount of creatine. Judging from the bladder the amount of creatinine in smooth muscle is different from that of striated muscles though these latter do not differ among themselves.

2. Does fatigue bring about a change in the amount of creatine or creatinine in an excised muscle? (a) Ten cats were killed and their extensors were removed. One from each cat was fatigued to exhaustion in a moist chamber by induced electric stimulation while its mate was hung in a moist chamber under similar conditions, but without stimulation. After the stimulated muscle was exhausted the total creatinine was determined in each. Four pairs of solei were treated in a similar manner. The results obtained were alike for the fatigued and resting muscle within the limits of error inherent in the method. Similarly there was no change found in the amount of preformed creatinine after fatigue under the same conditions.

3. Are the effects of fatigue upon the muscles of the intact animal the same as on the excised muscles? Three cats were decapitated after severe fatigue brought about by running in a revolving wheel. The total creatinine was determined as before. The results are shown in the last column of the table and indicate a loss of from 18 per cent to 30 per cent. That this loss extends to the bladder as well as to the muscles which were more obviously working is not contrary to recognized principles of fact and is, we think, capable of explanation.

	CREATINE + CREATININE; GRAMS PER 100 GRAMS OF MOIST TISSUE; RESTING MUSCLE	PREFORMED CREATININE; GRAMS PER 100 GRAMS OF MOIST TISSUE; RESTING MUSCLE	CREATINE + CREATININE; GRAMS PER 100 GRAMS OF MOIST TISSUE; ANIMALS FATIGUED IN WHEEL
Diaphragm	0.368 (23)	0.0044 (20)	0.288 (3)
Extensor	0.427 (23)	0.0048 (10)	0.324 (3)
Soleus	0.278 (23)	0.0042 (10)	0.228 (3)
Bladder	0.097 (10)	0.010 (9)	0.068 (2)

The cardio-skeletal quotient. W. L. MENDENHALL.

By means of faradic quantitative stimulation the relation between strength of stimulus necessary to excite contraction in the frog's ventricle and that necessary to excite contraction in the frog's gastrocnemius was investigated.

The heart was stopped by means of a ligature and the threshold was determined by means of electrodes which were thrust directly into the ventricular wall. The threshold of the gastrocnemius was determined by means of the same electrodes thrust directly into the belly of the muscle, the skin having been previously slit. The gastrocnemius was not curarized.

The threshold value of the gastrocnemius was divided by the threshold value of the ventricle. This ratio was found to be 0.17, and the name cardio-skeletal quotient was suggested for it. According to the evidence obtained the cardio-skeletal quotient is a body constant and indicates that the threshold of the frog's ventricle is approximately six times as great as the threshold of the gastrocnemius.

The use of the cardio-skeletal quotient as an index of the effects of physiological or pharmacological agents was indicated. An investigation using the quotient as indicated was reported. With the gastrocnemius kept in a constant or normal condition, an increase in the cardio-skeletal quotient would indicate an increase in the irritability of the ventricle: conversely a decrease in the cardio-skeletal quotient would indicate a decrease in the irritability of the ventricle. Accordingly it was shown that fatigue causes an increase in the irritability of the heart from 15 to 30 or 40 per cent, varying with the time which elapses after the electrodes are placed in the heart. It is greatest about forty-five minutes or an hour after the fatiguing process has been stopped.

Instances were reported wherein drugs caused decided changes in the irritability of the ventricle as shown by the cardio-skeletal quotient ranging from 0.04 to 0.76.

Hunger and appetite in fever. J. R. RUPP, J. MEYER and A. J. CARLSON.

In experimental fevers induced in healthy persons by the injection of sodium nucleate or excessive doses of typhoid vaccine, the gastric tonus and hunger contractions remain normal unless the body temperature exceeds 104°F., in which case they are depressed. In the fevers ranging from 101° F. to 104°C., the normal gastric hunger contraction may not give rise to the sensation of "hunger" but to an epigastric discomfort tinged with nausea, or "sick stomach." During the chill stage of the fevers, the gastric tonus and hunger contractions are depressed.

In experimental (non-infectious) fevers in dogs, the gastric hunger contractions remain normal, at least until the body temperature exceeds 104° F., when depression usually acts.

Bacterial toxins (exotoxins) or solutions of bacteria (endotoxins) appear to have no depressor action on isolated strips from the stomach wall.

The influence of temperature on the gastric hunger contractions of some of the lower animals. T. L. PATTERSON.

Within certain not very wide ranges of temperature the hunger movements of the frog's stomach fall within the limits of van't Hoff's rule. The hunger contractions of this animal are completely inhibited at 13°C. for low temperature and at 35°C. for high temperature. Raising the first temperature or lowering the second 0.5° or at the most 1° starts the hunger contractions again. In both the frog and the turtle the gastric hunger activity is proportional to the climatic changes in temperature within certain limits as is indicated by van't Hoff's rule.

Further observations on cerebral heat centers. ERNEST SACHS and P. P. GREEN.

Previous experiments stimulating the caudate nucleus faradically showed no temperature changes. Since then 93 experiments on cats and rabbits have been carried out. This paper also includes observations on over 150 craniotomies in human beings. There were 31 electrolytic lesions, 16 injection experiments, 40 irrigation experiments after the method of Barbour. In the electrolytic lesions, temperature rises were noted, but controls showed just as much rise. Injection of emulsion of cortex and caudate into the caudate showed similar temperature changes, but controls showed the same. The results of irrigation by the Barbour method were very variable and a rise of temperature with cold water and a fall with hot was not observed. Irrigation of the liver, using this cannula, gave similar results.

These experiments do not confirm the view that there is a cerebral heat center.

An application of Boyle's law and Avogadro's law to the oscillations of the manometer in clinical measurements of blood pressure. A. M. BLEILE.

Erlanger[1] has put forth an hypothesis bearing on the clinical measurement of blood pressure. This hypothesis practically states that the oscillations of pressure in the arm band, with a given volume pulse change, would be proportional to the pressure of the arm band; e.g., with a given volume pulse change, if the arm band pressure is at 100 mm., the pulse wave shown by the arm band manometer would be *only half as great* as it would with the same volume pulse but with the arm band pressure at 200 mm.

Upon testing this hypothesis by the help of a suitable physical model it is demonstrated that such is not the case. On the contrary, it is demonstrated that oscillations of volume occupied by given mass of gas produce inversely proportional oscillations of absolute pressure.

Or, in other words, the absolute pressure of a given mass of gas is inversely proportional to its volume.

In short, it is another way of stating Boyle's law, that the absolute pressure of a gas is proportional to its concentration.

Therefore, the results of the present work are in harmony with Boyle's law but are contrary to Erlanger's hypothesis.

The influence of an oxidizing substance on the catalase value of the blood. AARON ARKIN.

In previous work the author has demonstrated that certain oxidizing substances (sodium iodoxybenzoate) stimulate the production of antibody in immunized animals, as was shown by Hektoen for dogs immunized with ox corpuscles. The author also found that the above substance inhibits the tuberculin reaction in tuberculous animals, as shown by Amberg for other local allergic reactions, and concluded that the stimulating effect on antibody production was due to a stimulation of oxidation at the sites of antibody production. In an attempt to analyze this action a study has been made of the effect of oxidizing substances on the catalase value of the blood and tissues. With the assistance of E. B. Fink I have found that sodium iodoxybenzoate does not affect the catalase value of the blood of rabbits. Other oxidizing agents studied by me have no effect. The study of the effect on the tissues of immunized animals is now under way.

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[1] Erlanger: This Journal, 1916, xxxix, 401.

The vaporization of water from skin and respiratory passages in health and disease. EUGENE F. DU BOIS and G. FABIAN SODERSTROM.

The water elimination has been determined in 300 experiments made on patients and normal controls in the Sage respiration calorimeter. All observations in which the relative humidity changed more than 10 per cent in three hours have been excluded from the averages since large amounts of water vapor can be absorbed or given off by the bed and clothing with a changing humidity. This leaves 175 experiments made under standard conditions, the air temperature between 22 and 24°C.; relative humidity, 25 to 50 per cent; and the subjects dressed in shirt, pajamas and two pairs of socks.

Thirteen normal men gave off on the average 29 grams of water an hour and 24 per cent of the calories were lost through the vaporization of water, with a range of plus or minus $\frac{1}{10}$. Average results very close to this figure were given by men after large meals of glucose or protein, boys twelve to thirteen years old, very old men, men with legs cut off, dwarfs, patients with pernicious anemia, cardio-nephritic disease and diabetes. The percentage of heat lost through vaporization was increased in typhoid patients with falling temperature and in a few cases of hyperthyroidism.

Some physiological disturbances induced in animals by nitrobenzol fumigation. M. DRESBACH and W. L. CHANDLER.

This paper deals, as a preliminary report, with the effects of nitrobenzol on animals when it is given by inhalation, under conditions in which disturbing factors, such as impurities, CO_2, excessive moisture and temperature variations are reduced to a minimum.

Nitrobenzol acts mainly on the central nervous system and blood. The present study is concerned with the former only. The symptoms vary with different species of animals and with individuals of the same species. In rabbits, cats and rats the dominant symptom is depression and paralysis; in frogs these symptoms alone are seen; in dogs the action is more complex. Rabbits and guinea pigs are very resistant to the poison, but they succumb quickly once the symptoms develop. Dogs may show signs of poisoning within a few hours after breathing the vapor, or not until several (even four) days after the inhalation. The time will depend upon the amount of the benzol taken in. One of the first effects is vomiting. This is followed soon by loss of coordination of muscles. The hind legs are the first, usually, to be affected; then follow the fore leg muscles and those of the neck, jaw and trunk. Extensor rigidity is the most prominent condition; opisthotonus may be very marked. The attacks may last variable lengths of time, some times for hours. In dogs and guinea pigs typical "swimming" movements are often seen, persisting for shorter or longer periods. In dogs there is practically always nystagmus, and we have seen other evidences of cerebellar stimulation. Likewise, there is dilation or contraction of one or both pupils. Consciousness is present till a late stage. Digestion is interfered with from the start and the animals usually refuse food and drink, though they can often be persuaded to take liquids between attacks of the spasms.

Recovery may take place within a week or ten days following the onset of the intoxication, or the animal (dog) dies, apparently from respiratory failure. We have not seen the circulation fail first.

The experiments so far made indicate that nitrobenzol stimulates and then paralyzes motor centers of skeletal muscles. The circulation and respiration in dogs are but little affected, unless violent convulsions ensue. The effects upon the blood have not been sufficiently studied, but it is known that profound changes in the oxygen carrying capacity and in the morphology and number of the different corpuscles may occur.

Physiological effects of ethyl alcohol when injected into the rectum with special reference to the gaseous exchange. T. M. Carpenter.

The respiratory exchange of four healthy medical students before and after injection of alcohol into the rectum was studied by means of a face mask and the Tissot respiration apparatus. For control studies 0.6 per cent sodium chloride solution was used. The composition of the expired air was determined by the portable Haldane gas analysis apparatus. Simultaneous graphic records were secured of the pulse and respiration rates as well as of the extraneous muscular activity. The concentrations of alcohol injected were 5.0, 7.5 and 10 per cent. The amounts given were from 200 to 750 cc. Usually the absolute weight of alcohol was 25 grams.

When the respiratory quotient was about 0.85 before 25 grams of alcohol was given, it fell to 0.80 two to three hours after the injection; when the respiratory quotient was 0.80, it fell to 0.77 in about the same length of time. This depression of the respiratory quotient did not occur in all cases. In a few experiments the oxygen consumption was raised about 10 per cent. within three hours after the alcohol. In several instances the pulse rate increased from three to five beats per minute over that of the period before alcohol was given.

Reflex control of gastric vagus-tonus. F. T. Rogers.

Dogs, in which the splanchnic nerves have been sectioned and with gastric fistulae, may show hunger contractions after decerebration (Sherrington's method) following a fast of two or three days. Transection of the brain stem must be done under *deep* anesthesia. Unless the dog again breathes normally and unless the pulse rate and strength are nearly normal, the hunger contractions will not appear. Respiratory or cardiac disturbances indicate a quiescent stomach. Pressure effects on the medulla do not seem to stimulate the gastric centers.

Carlson has called attention to the fact that the hunger contractions are very easily inhibited by various reflexes but in no case is there a reflex augmentation of these contractions or of gastric tone.

In a dog, prepared as indicated above, stimulation of the central end of one vagus nerve with the other nerve intact, is followed by reflex spasmodic contractions of the entire stomach. This effect may be obtained by stimulating the nerve either in the neck or below the diaphragm. In the absence of the splanchnic nerves reflex augmentation of gastric tone and contractions is possible. The vagus contains both the sensory and motor paths of this reflex. This suggests that vagus tonus, with reference to the stomach, may be a reflex effect similar to that of muscles.

An instance of apparent anesthesia of a solution. E. NEWTON HARVEY.

Luminous bacteria will cease to light in definite concentrations of an homologous series of alcohols, and if the alcohol be removed or diluted light again appears. The concentrations which narcotize in one hour are: methyl alcohol, 2 m.; ethyl, 1.5 m.; propyl, 0.25.; butyl, 0.08 m.; amyl, 0.03 m.; capryl, 0.002 m. The series is similar to that found in narcosis of other cells and brings the anesthesia of light production in line with that of muscle contraction and other physiological processes. The preparation of luminous extracts of luminous bacteria is practically impossible. Many luminous animals, however, secrete luminous substances; the small ostracod crustacean, *Cypridina*, forms a brilliant clear luminous secretion whose light is perfectly homogeneous. The secretion contains both photogenin and photophelein, the substances necessary for the production of light, and it may be filtered through a porcelain or siliceous filter candle and is then still luminous. The light producing substances are therefore in true or colloidal solution. If ethyl alcohol is added to the porcelain filtrate, the light disappears when about 20 per cent alcohol is reached and on diluting, the light returns. With butyl alcohol, less than 8 per cent is needed. As no precipitate forms, we are not dealing with a precipitation phenomenon, but with a reversible inhibition of light production in a solution akin to anesthesia.

Minimal variations in spinal reflex thresholds. EUGENE L. PORTER.

The object of this investigation was the study of and control over some of the variables encountered in measuring reflex thresholds in the spinal cat, and in particular the variations in threshold of the flexion reflex were to be compared with those of the nerve muscle preparation. Spinal cats had tibialis anticus muscle arranged for recording myographically, when reflex contraction was elicited by stimulation of the posterior tibial nerve; subsequent stimulation of the distal end of the cut peroneus nerve gave direct nerve-muscle records from the same muscle. A modification of Lucas' liquid electrode was used in place of the platinum wire type, to avoid local impairment of the nerve from compression, and to prevent alterations in resistance at the point of stimulation due to the presence or removal of liquid. This electrode consists of a short glass tube, constricted at one end, into which the ligated nerve is drawn; over the other end is pushed a rubber tube, whose lumen 1 cm. from the glass tube is closed by a glass ball through which passes a platinum wire. Within the glass and rubber tubes the nerve is surrounded by a thin paste of kaolin in Ringer's solution. Induction shocks sent into the platinum wire spread through the kaolin-Ringer paste, stimulate the nerve at the constricted neck where current density is greatest, and leave the animal at an indifferent electrode on the fore-limb. Determinations of threshold were made approximately once a minute for periods an hour long, using a coil calibrated by Martin's method. During an hour both the reflex and nerve-muscle preparations were found to vary as much as 2 Z-units but at the end the threshold might be either lower or higher than at the beginning. Alterations of threshold between successive readings were from two to three times as great for reflex as for nerve-muscle, and the direction of change toward a higher or lower threshold was reversed more often for reflex than for nerve-muscle. Comparisons of flexion with crossed-extension showed that flexion thresholds varied less than those for crossed-extension.

Possible periodic variations in the extent of the knee-jerk in women. JESSIE L. KING.

Repeated attempts have been made to demonstrate by physiological and psychological tests that the life-processes of women have a rhythmical movement which centers about the menstrual period. Except as concerns the slight constant variations in pulse and temperature, these attempts have failed. Possibly a variation in the tone of the nerve-centers, as well as the rise in temperature may account for the feeling of discomfort frequently experienced by normal women immediately preceding and at the onset of the menstrual period.

Using the knee-jerk as an index of such tone, a series of daily observations was made on a group of eleven normal young women, over periods varying from one to five menstrual cycles. The observations covered twenty-nine periods. A chart showing the daily averages for one subject was submitted.

The evidence pointed in the direction of the following conclusions—that a period of hyperexcitability immediately precedes or accompanies the onset of the menstrual period; that this is followed by a decline in excitability which continues for a few days after the menses has ceased; and that there is then a tendency for it to rise to a slightly higher level than the preceding during the intermenstrual interval.

The combination of thrombin by the antithrombin of the blood serum. H. S. GASSER.

If very clear oxalated plasma is heated to 55° to remove its fibrinogen, the coagulation process may be initiated by optimum calcification and the stages in the conversion of prothrombin be studied quantitatively from the start. If the amount of tissue extract present is so small that all the prothrombin is not converted to thrombin then the amount of thrombin that can be produced is formed rapidly and disappears rapidly, going into combination with antithrombin. Quantitative determinations show that the curve of free thrombin is practically the inverse of the combined-thrombin curve. The system soon comes to equilibrium, no more prothrombin is converted to thrombin, therefore there is no evidence that the action of tissue extract is catalytic. This equilibrium is very readily upset, however, by addition of tissue extract, or by simple dilution. The thrombin in combination with antithrombin is in an irreversible combination; processes liberating the thrombin also destroy the antithrombin. The rapidity with which thrombin is combined by antithrombin explains satisfactorily why thrombin does not cause thrombosis when injected intravenously and is to be regarded as an important agency in maintaining the fluidity of the blood.

The inhibitory effect of adrenalin upon the sphincter muscle of the iris. DON R. JOSEPH.

Adrenalin solutions of various concentrations when applied to the sphincter pupillae after either partial or complete excision of the muscle cause a prompt and unmistakable relaxation. This relaxation is maximal following the use of stronger adrenalin solutions (1: 1000 to 1: 100,000). Very dilute solutions (1:10,000,000) on the other hand, produce only a partial relaxation. The duration of the effect varies directly with the concentration of the solution used; after 1: 20,000,000 recovery may be complete within two to five minutes, whereas after 1: 1000 two hours or more may be necessary for recovery. The sphincter muscle of the following irises was tested: bovine, swine, sheep, goat and human. The changes in sphincter-length were recorded graphically.

These experiments demonstrate that the sphincter muscle of the iris is capable of responding to a suitable stimulus in a sense that is exactly the opposite of shortening (contraction), namely by lengthening (relaxation). In its response to adrenalin the sphincter of the iris resembles the intestinal strip.

The transformation of the plasma clot. GEORGE A. BAITSELL.

It has previously been shown by the author[1] that in tissue cultures and in wound healing in the skin of frogs a fibrous tissue which gives evidence of being identical with normal connective tissues may be formed by a direct transformation of a plasma clot. In an endeavor to analyze this reaction more fully, plasma clots made from centrifuged blood plasma of the frog have been subjected to various conditions of tension and pressure. The results obtained show that with the aid of these mechanical factors it is possible to directly transform a typical fibrin net into a fibrous tissue. By varying the conditions it is possible to obtain preparations which will show various stages in the transformation ranging from an almost typical fibrin net to a fibrous tissue composed of bundles of wavy fibers such as are characteristic of various types of normal connective tissues. This newly formed fibrous tissue when stained with Mallory's connective tissue stain (modified according to Mall) takes the typical blue color the same as do regular connective tissues.

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[1] (a) Journ. Exper. Med., 1915, xxi, 455–479; (b) Journ. Exper. Med., 1916, xxiii. 439–456.

Changes in the percentage composition of muscle protoplasm during prolonged fasting with work. C. W. Greene.

The Pacific salmon, Oncorhynchus tschawytscha, was used for the determinations presented in this series of experiments. This species of salmon, as is well-known, feeds in the ocean until maturity and then makes the journey to the spawning grounds in cold fresh water streams wholly without eating, a natural fasting period. The journey takes three to four months, and there is expenditure of a great amount of muscular energy during the migration. The source of this energy has been traced by the method of quantitative chemical analyses of the great classes of substances constituting the muscle tissue.

The salmon stores its fat among its muscle fibers and within certain classes of fibers. This storage fat amounts to from 15 per cent to 20 per cent of the wet weight of the muscle, in certain particular muscles as much as 30 per cent. At the end of the migration the excess of fat is reduced to an average of 2 per cent to 2.24 per cent. It has been shown that a large proportion of the gross mass of muscle disappears during the migration fast. The stored fat has been considered as a superload in the determination of the protoplasmic changes. When figured on the assumption that the tissue exclusive of fat may be legitimately considered as muscle protoplasm rather striking compositional changes appear.

Comparing the three collecting regions, namely, the mouth of the Columbia River, an intermediate point at 700 miles up stream, and at the spawning grounds, the following changes are revealed: The water content of the fat-free tissue increases—74.7 per cent, 77.0 per cent and 81.5 per cent, an increase in total water content of about 9 per cent during the journey. The proteins decrease—20.2 per cent, 18.2 per cent and 14.0 per cent, a relative decrease of 30 per cent. The organic extractives increase—2.9 per cent, 3.3 per cent and 3.1 per cent. Migrating fish are most active at the 700 mile station, which doubtless accounts for the higher percentage of organic extractives obtained at this point. The ash remains practically constant throughout the entire cycle of the migration fast.

Further studies on the effect of adrenalin upon muscular fatigue and limb circulation. CHAS. M. GRUBER.

The experiments were performed exclusively upon cats, either under urethane or ether anesthesia. The drop method was used in recording the circulation. The limb was either under normal conditions, except that the nerve was cut and a cannula placed in the blood vessel leading from the muscle under observation or perfused with warm Ringer's solution through the femoral artery.

In all experiments when the nerve was cut, adrenalin slowly injected in small doses 0.5 cc. to 2 cc. of a 1: 100,000 solution produced an increase in the height of muscular contraction, with a concomitant decrease in the rate of flow of fluid through the vessels. This decrease in the intact animal was passive in action, due to the fall in blood pressure. But in the perfused limb small doses 1: 100,000,000 produced active vasoconstriction.

Under experimental conditions in which there is paralysis of the nervous system to the muscular vessels adrenalin does not better fatigue by bettering the circulation, but it betters the height of muscular contraction by its action upon some substances produced during fatigue or upon some substance utilized by the muscle during its contraction.

In a fatigued muscle in which the nerve is intact adrenalin has a twofold effect: that of bettering the circulation, and that, possibly, of direct chemical action similar to that found in a muscle in which the nerve is cut.

During the development of rigor mortis changes take place in the muscle similar to those that take place in fatigue. Adrenalin was found to have a potent action upon muscles undergoing rigor mortis from one to four hours as tested by muscular contraction.

Anti-neuritic substances from egg yolk. H. STEENBOCK.

By means of neutral solvents there was prepared a water-acetone soluble fraction from egg yolk which in small doses by intraperitoneal injections was able to cure a pigeon suffering from polyneuritis. The anti-neuritic principle was found stable to concentrated hydrochloric acid at 98° and to concentrated alkalies at room temperature, but readily destroyed by dilute alkalies at the boiling temperature. Phosphotungstic acid precipitated it very incompletely. It was not adenine.

On the mechanism of blood coagulation. G. H. A. CLOWES.

A suitable citrated plasma filtered through a bougie does not clot on the addition of $CaCl_2$ alone, but if one in a hundred thousand of boiled brain lipoid is added, the filtrate clots with $CaCl_2$ almost as readily as does the original plasma. The filtrate without addition of lipoid clots far more readily with thrombin than does the original plasma, the speed of coagulation being if anything retarded by the addition of lipoid. If brain lipoid is added in excess to such a filtrate without addition of $CaCl_2$ and the mixture filtered a second time, the filtrate obtained does not clot with $CaCl_2$ alone, clots less readily than the first filtrate with $CaCl_2$ and lipoid and more readily with thrombin. Mixtures of $CaCl_2$ and lipoid or $CaCl_2$ and prothrombin which are toxic for mice on intravenous injection retained their toxicity indefinitely, but a mixture of $CaCl_2$, lipoid and prothrombin in the proportions necessary to cause coagulation exhibits a gradually diminishing toxicity.

The action of brain lipoid is destroyed by the addition of sufficient iodine. Lecithin exerts no effect. Unsaturated lipoids appear to play a positive rather than a negative rôle in blood coagulation.

NaCl at a concentration of 0.35 to 0.4 M destroys living protoplasm in marine organisms or mice, exerts an inhibiting effect upon blood coagulation, breaks down emulsions of oil dispersed in water by Na soap, precipitates soap jellies and soap and lipoid suspensions, and the ratios in which the effect exerted by NaCl and other salts may be counteracted by $CaCl_2$ at varying concentrations appear to be approximately the same in blood coagulation and many of the above systems and to correspond with Loeb's data on antagonistic effects exerted by electrolytes on protoplasm and the writer's observations on interfacial soap films determined by the drop method.[1] The relation between prothrombin and thrombin presents a striking analogy to that between soaps like sodium oleate, which being soluble in water but not in oil promote the formation of emulsions of oil in water, and soaps like calcium oleate, which being soluble in oil but not in water promote the formation of emulsions of water in oil. The consideration of coagulation as a possible reversal of phase relations in plasma under the influence of Ca, analogous to that in emulsions, may offer an explanation for such phenomena as the durability of prothrombin in citrated plasma, its inability to function without calcium and lipoid, the high coagulating effect of the nascent thrombin formed from calcium, lipoid and prothrombin in the presence of fibrinogen, the extremely rapid deterioration of thrombin when present in the water phase, presumably as a result of surface adsorption, its stability so long as it remains a portion of the non-aqueous phase and its ability to function although somewhat slowly in the presence of an excess of citrate.

Recovery experiments following removal of the Sino auricular node in the dog's heart. J. A. E. Eyster and W. J. Meek.

The sino-auricular node was sewed off in seven dogs, in three additional animals it was cut away. The operations were performed aseptically and the animals allowed to recover. Before, and at varying periods of time after the operation, electrocardiograms (lead II) were taken to determine the type of cardiac rhythm. In four experiments, the reaction to muscular exercise and to atropine sulphate before and after the operation was also determined. The experiments varied in duration from 48 hours to 64 days following the operation. In most cases, at the termination of the experiment, the chest was opened and the seat of impulse formation determined by initial negativity with the string galvanometer. Eight of the animals showed periods of auriculo-ventricular rhythm following the operation. In all, however, there subsequently developed a rhythm in which there was a definite, though usually somewhat shortened, auriculo-ventricular interval. In one experiment the seat of the permanent rhythm was definitely within the coronary sinus portion of the auriculo-ventricular node. Others showed the resumption of an apparently normal sino-auricular rhythm, preceded in two by evidences of partial sino-auricular heart block. Histological examinations will have to be made before definite statements can be given as to the relation of the seats of permanent impulse formation to possible remnants of the sino-auricular node.

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[1] Journ. Phys. Chem., 1916, xx, 407.

The perfusion of the mammalian medulla: the effect of carbon dioxide and other substances on the respiratory and cardiovascular centers. D. R. HOOKER and D. W. WILSON.

To determine the effect of various acids and alkalies on the mammalian medulla, perfusion experiments were carried out on preparations similar to those described by one of us in which an isolated circulation is maintained artificially through the head and neck of the animal. Various materials were added to portions of the perfusing blood and the effect on the respiratory and cardiovascular centers noted by recording the movements of the epigastrium and the pressure changes in the femoral artery. In preliminary experiments sodium hydroxide added to blood caused complete inhibition of respiratory movements and hydrochloric acid caused marked stimulation. Sodium bicarbonate caused an increase in amplitude and decrease in rate of respiratory movements. Further experiments were more carefully controlled by determinations of the hydrogen-ion concentrations of the specimens used.

Portions of blood in which the P_H was decreased from 7.4 to 7.2–7.3 by introducing hydrochloric and lactic acids, showed no apparent differences in stimulating action on the respiratory center of the same animal.

Portions of blood through which 5 per cent CO_2 had been passed were compared with blood to which HCl had been added and the free CO_2 shaken out, the resulting P_H being the same in both cases. Alternate perfusions of these solutions through the same preparation resulted in a much greater stimulating action of the blood containing the excess of CO_2. These experiments would indicate a specific action on the part of carbonic acid on the respiratory center.

Observations on immunity of monkeys to experimental poliomyelitis. EDWARD C. ROSENOW and GEORGE W. WHEELER.

Apparent immunity was conferred upon three monkeys by inoculations, which were not followed by obvious evidence of infection. One of the immune animals had previously received an intracerebral injection of plemorphic streptococcus, the second had received emulsions of the central nervous system of rabbits paralyzed by cultures of this streptococcus, and the third had received virus in the usual way. One monkey injected with green-producing streptococci from sources other than poliomyelitis was not immune, and one monkey injected with virus from monkey which proved not to have poliomyelitis was not immune. Three normal controls developed poliomyelitis in the usual time.

Pulse velocity and blood pressure. An attempt to determine the variations of elasticity in the walls of living arteries. PERCY M. DAWSON.

Many physiologists have come to regard the pulse pressure as an index of the systolic output of the heart (see Dawson and Gorham, 1908). It is however obvious that a pulse pressure of say 50 mm. when the mean pressure is 100 has not the same significance as the same pulse pressure when the mean pressure is 130, unless the coefficient of elasticity of the blood vessels remains constant, which is probably not the case.

Variations of the coefficient of elasticity of the arteries outside the body have been carefully studied (see MacWilliam, 1904, whose observations have been confirmed in great part by Higgins, 1908). But we have no certainty as to the variations which this coefficient undergoes in the living animal. As an index of the variations of arterial elasticity in the living animal one may take the variations in the rate of transmission of the pulse wave. Then if the vessels behave in the body as they do when outside it, one might expect that at the very least a rise in mean pressure would be accompanied by an increase in the velocity of transmission of the pulse wave.

In 1908 the writer, assisted by Lemuel W. Gorham, determined the rate of transmission of the pulse wave in dogs while the blood pressure was being raised and lowered by stimulation of the vagus and of various sensory nerves (central end of vagus and of saphenous). This study showed that the correspondence between the blood pressure and the rate of transmission was by no means close. For example the curve of elasticity would, after rising steadily for a while, in correspondence with the rise in pressure, suddenly fall or begin to oscillate up and down. Consequently if one should determine the rate of transmission for a pressure of say 60 mm., he might find ten minutes later that this value corresponded to an entirely different pressure. These experiments were repeated and confirmed in 1916 by Edwin G. Bloor and Fred J. Hodges, working under the direction of the writer.

The results of those experiments were not however accepted by us because of our lack of confidence in the method of recording. This was by means of two Hurthle manometers which gave curves upon which it was sometimes impossible to identify the beginning of the up-stroke of the pulse wave. It was not until the previous results were again confirmed by experiments in which the segment capsules were employed that publication seemed justified. Such results were obtained by the writer assisted by Hartwik M. Stang and Fred J. Hodges.

We conclude that in so far as the velocity of the pulse rate may be regarded as an index of arterial elasticity, it indicates that in the living animal the coefficient of elasticity is irregularly variable and it is furthermore suggested that this variability may be due to changes of tonus of the vascular walls.

Does the myelin sheath function as an electric insulator broken only at the nodes of Ranvier? J. F. McClendon.

Many hypotheses have been advanced concerning the function of the myelin sheath. The supposition that it is an electric insulator is not at all new, but may seem out of harmony with the fact that the nerve may be stimulated electrically throughout its length. Through repeated confirmations of the All-or-none Law it has been shown that not all of the fibers in a nerve are stimulated by a minimal stimulus, which suggests the possibility that the current enters and leaves the nerve fiber at a node of Ranvier and that those fibers are stimulated by a minimal stimulus whose nodes are favorably placed. It is often asserted by teachers of physiology that a nerve can not be stimulated by means of a current passing at right angles to the course of the fibers (unless spreading alters the course of the current), which also suggests that the current enters and leaves by the nodes, since more nodes are affected by a current passing lengthwise than by one passing crosswise. In attempting to collect further data on this question the nonmedullated nerves of Limulus were stimulated by minimal currents, and it was found that electrodes the same distance apart, equal stimulation was the result of the current passing crosswise as of the current passing lengthwise, which is in harmony with the hypothesis discussed.

The oxygen pressure necessary for cellular activity. Montrose T. Burrows.

In an early study the author had noted that the oxygen necessary for cellular activity in a tissue culture comes from the air chamber of the hollow ground slide in which the culture is suspended. To reach the cells it must diffuse from this space into the culture medium which bathes them. The cultures were made by placing a small fragment of tissue, 1 mm. in diameter, in a layer of plasma on the surface of a cover glass. The cover glass is inverted over a hollow slide so that the medium and tissue hang in the air chamber. The cover is sealed to the slide with vaseline and paraffine to prevent drying.

In the present study a special type of culture chamber has been constructed. It is so arranged to allow one to pass into it any gas or combination of gases. The air chamber of this culture is large, 40 to 60 times that of the ordinary culture in which growth may continue actively for several days. This culture chamber is made from one piece of glass and it can be sealed by fusing. Tests to determine the effect of pure oxygen and partial pressures of oxygen on the growth of the cells have been made. The partial pressures of oxygen were obtained by diluting pure oxygen with the nitrogen gas. The tissues used have been fragments of heart muscle and skin of chick embryos and foetal chickens. The medium is blood plasma, prepared from the blood of adult chickens.

There is a small error in the measurements of the partial pressure of oxygen.

Including this error the results have been considered interesting. The cells grow actively in pure oxygen. The growth is not greater in an atmosphere of pure oxygen than in an atmosphere containing 9 or 10 per cent of oxygen. A small growth is seen when the partial pressure of oxygen is as low as 6.6 per cent, 45.6 mm. of mercury.

The cause of cataract. W. E. Burge.

In the production of cataract there are at least two factors, one, the short wave lengths of the spectrum, the other, inorganic salts such as those of calcium, silicon, etc. which are found to be greatly increased over the normal in cataractous lenses. By increasing either of these factors separately far beyond what the eye would ever be subjected to an opacity of the lens or cataract can be produced, e.g., cortical cataract can be produced by immersing the lens in a 5 per cent solution of calcium chloride, and nuclear cataract by immersing the lens in a 15 per cent solution of potassium chloride. By increasing these two factors simultaneously an opacity of the lens or cataract can be produced under conditions that might easily prevail in the body, e.g., if the lens s exposed to the short wave lengths of the spectrum it causes a molecular rearrangement in the protein so that a very weak solution of calcium salts will precipitate it and hence produce an opacity or cataract. In this respect the inorganic salts seem to act in a manner comparable to that of a developer in photography.

Others presented papers, as follows:

The fate of intravenously injected dextrose. J. J. R. Macleod.

Primary scrotal syphilis of the rabbit and its utilization in chemotherapeutic experiments (with lantern demonstration). W. H. Brown and L. Pearce.

The action of some optic isomers on the ureter. D. I. Macht.

The time relations of the fundamental heart sounds. Carl J. Wiggers and A. Dean, Jr.

A comparison of the effects of food and of caffeine on work in the athlete and an untrained subject. C. B. Root and H. Curl.

On the toxemia of intestinal obstruction. L. R. Dragstedt, W. Burby and A. J. Carlson.

The mechanism of the regulation of the intra-abdominal pressure. Helen C. Coombs.

Some considerations of the isolated bone marrow. Cecil K. Drinker, Henry A. Kreutzman and John R. Paul.

Labyrinthine reactions in kittens (with demonstration). A. L. Prince.

Some observations on the conditions of activity in the adrenal glands. W. B. Cannon and H. F. Pierce.

Endermic reactions. J. D. Pilcher and T. Sollman.

The active constituent of the thyroid; its isolation, chemical properties and physiological activity. E. C. Kendall.

The effects of temperature change on rhythm in the human electromyogram. Alexander Forbes and W. C. Rapleye.

The so-called experimental streptococcic poliomyelitis (with lantern demonstration). C. G. Bull (by invitation).

The attenuation of the toxic action of KSCN *by elevated temperature.* R. A. Spaeth.

Bacteriologic observations in experimental poliomyelitis of monkeys. Edward C. Rosenow and Edward B. Towne.

Papers read by title, as follows:

The inorganic composition of thoracic duct lymph. A. B. Macallum.

Preliminary report of cardiogram and blood pressure records, showing the effect of music. Ida Henrietta Hyde.

The hydrolytic products of chitin. S. Morgulis.

Demonstrations as follows:

A signal magnet which writes either upwards or downwards. Worth Hale.

In this magnet, which is useful in recording time intervals, etc., use is made of a double magnet and double armatures. One of these armatures is extended as a writing point, the other serves as an arrest for the recoil produced by the spring when the current is not acting. The armature which thus serves as an arrest is held stationary by its spring against a fixed block. When the electrical current passes to one magnet the writing armature moves in one direction, and when to the other magnet it moves in the opposite direction.

An improved lever for frog's heart and muscle strips. A. H. Ryan.

The essentially new feature in this lever is the use of needle points as bearings. By this method greater sensitivity is obtained and at the same time lost motion is eliminated.

A saddle is made which carries a straw. On one side of the under surface of the saddle is a conical depression, and on the other side is a wedge shaped slit running at right angles to the axis of the straw. The conical depression and slit each rests upon a needle. These needles are mounted in a support which has attachments for two adjustments by which the lever may be made to write parallel to the recording surface; and by which the lever may be made to write with more or less pressure upon the recording surface. The lever may be used for frog's heart tracings or isolated strips. The detachable arm which supports the strips is hollow to permit of passing gases or liquids into the medium surrounding the strip.

A crude form which could quickly be carved out of cork was also demonstrated.

The inhibitory effect of stimulation of the central end of the vagus nerve upon the contractions of an active respiratory muscle in the chicken. A. L. MEYER.

The innermost abdominal muscle in the fowl when properly exposed is observed to contract during each expiration. It undergoes a sudden relaxation just prior to the onset of inspiration.

The central end of the vagus was stimulated with an induced current. The muscle remained quiescent in a state of relaxation, while the thorax assumed an inspiratory position. This result was obtained many times and without fail during the demonstration. The phenomenon here presented is another instance of "contrary innervation" (Meltzer) or "reciprocal innervation" (Sherrington). This method, it seems, may be useful in demonstrating the subject of contrary innervation to students.

A gas analysis apparatus. YANDELL HENDERSON.

A simple, easily operated and comparatively cheap analyzer was shown, and the methods of determining the composition of the alveolar air, the respiratory metabolism and the blood gases CO_2, oxygen and "oxygen deficit" were demonstrated. While the apparatus is designed primarily for students in a laboratory course it is found to be sufficiently accurate for most purposes of research.

The motion picture as an aid in teaching physiology. J. A. E. EYSTER and WALTER J. MEEK.

Motion picture films were exhibited showing certain typical physiological experiments. It is believed that these are of value as an aid to physiological teaching in the following instances. (1) As a supplement to certain demonstrations. There are certain important aspects of many demonstrations which are difficult to exhibit to a group or class by the ordinary methods. (2) To serve for demonstration of certain experiments which (a) are too long to be carried out in the classroom during the regular class period, (b) in which the typical results are obtained only at certain times under certain conditions, or (c) which are too time consuming or too expensive to involve repetition before each class. (3) To assist in the analysis of certain rapid movements. One of the films shown was that of the exposed beating mammalian heart, taken at a rate of exposure of 80 per second and projected at the normal rate of 16 per second. (4) For demonstrations before elementary classes where the more complex demonstrations involve too much time or expense.

It is not believed that this method will do more than supplement already existing methods of teaching physiological science.

Motor phenomena of the stomach, pylorus and cap observed Roentgenographically. LEWIS GREGORY COLE.

Each portion of the gastro-intestinal tract has a characteristic Roentgenological appearance: The ileum by its coils, the jejunum by its flocculent appearance, the duodenum by its circular rings or its shape, the stomach by its characteristic shape.

That portion of the tract just beyond the pyloric sphincter, previously known as the first or ascending duodenum, has the characteristics of the stomach rather than the duodenum. Embryologically, together with the stomach, it is derived from the foregut. Histologically it contains the glands of Brunner. Ferments from the glands of Brunner are active only in an acid medium; therefore this region is rationally acid. Surgically 95 per cent of ulcers occur in this region. Roentgenologically its contour corresponds with the pyloric end of the stomach, and the manner in which it surmounts the pyloric end of the stomach resembles a cap; therefore the term "cap of the stomach" or "pilleus ventriculi" has been applied to this segment of the gut.

The cap is separated from the pyloric end of the stomach by a clear cut space of about $\frac{3}{16}$ of an inch; this indicates the pyloric valve, and the size of the lumen of the valve can be determined.

A meal composed of mixed foods and fluids begins to be evacuated immediately; certainly before ingestion of a full meal is complete.

The gastric motor phenomenon is complex rather than simple and is composed of the progression of the peristaltic waves pyloruswards and a systole and diastole, and it may be considered in the form of a cycle similar to the cardiac cycle. The stomach assumes a series of shapes and eventually assumes the same shape that it was approximately fifteen or twenty seconds earlier. This is referred to as a gastric cycle. If a peristaltic sulcus travels from its origin to the pylorus during one cycle it is a one cycle type of gastric peristalsis. If it requires two, three or four cycles for a sulcus to travel from its origin to the pylorus this is respectively referred to as two, three or four cycle type of peristalsis. During approximately seven-tenths of each gastric cycle all of the peristaltic sulci synchronously increase in depth; this is gastric systole. During approximately three-tenths of each cycle these sulci synchronously relax; this is gastric diastole. During the systole of each gastric cycle, when the pressure against the proximal surface of the pyloric valve is greatest, the pyloric sphincter is partly opened, either actively or passively, and the chyme is propelled into the reservoir cap; and during the stage of diastole when the gastric pressure is diminished the sphincter may be closed to prevent the chyme from falling back into the stomach.

There is no Roentgenological evidence in man of a periodical opening and closing of the pyloric valve independently of each gastric cycle, as described by Cannon in the American Journal of Physiology, Volume XX, no. 2. If such a phenomenon does not exist, how can it be acid controlled?

The duodenum is replenished from the reservoir cap where the finishing touch of proteid digestion is applied to a small amount of chyme. The cap is evacuated by a broad peristaltic wave, independent of the gastric peristalsis, which forces the chyme through the duodenum in "finger-like masses." The frequency of this wave may be controlled by an alternating acid and alkalin reaction in the descending duodenum.

Demonstration of Nissl preparations indicating an absence of chromatolytic changes in the central nervous system of the woodchuck (Marmota monax) during hibernation. A. T. Rasmussen.

The whole problem of the correlation of structural changes in nerve cells with degrees of activity, having been re-opened by the recently reported findings of R. A. Kocher, it is interesting to note the apparent absence of demonstrable changes in the tigroid bodies of nerve cells of animals which have been profoundly torpid almost continuously for months, during which time no food or water was available. Similar results have been reported in other animals, though contrary conditions are also on record.

Killing, fixing and embedding were carried out as uniformly as practicable. Homologous sections from an entire series of animals were placed together on the same slide for staining or an entire series of slides was carried through the reagents in a basket which would contain the entire lot. Two series of animals, involving a total of 21 animals, 9 of which were sacrificed during hibernation, were investigated.

Glycogen in the blood vessels of the liver. G. Carl Huber and J. J. R. Macleod.

Several microscopic slides were shown of sections of liver, removed immediately after death by stunning, from rabbits previously fed with carrots. The pieces of liver after removal from the animal were immediately fixed in absolute alcohol and later embedded in paraffin, cut in sections of 5 mm., and stained by a somewhat modified Best's carmine method. By this means the glycogen is stained a deep red and the nuclei of the cells can easily be counterstained.

Certain of the preparations were from animals that were in a perfectly normal condition up to the moment of stunning. Others were from rabbits in which, three or four hours previous to the stunning, the medulla had been punctured with a needle and the animal thus rendered diabetic. By comparing slides from the two groups of animals it was shown that in normal rabbits the glycogen is present only in the liver cells and never in the sinusoids or sublobular veins, whereas in the livers of diabetic rabbits red-staining glycogen masses are often present in the latter vessels. It is not of course claimed that, because this material in the blood vessels stains exactly the same as the glycogen of the cells, it is necessarily of the same chemical nature.

It was also pointed out by the authors that a very large number of preparations have been examined in order to exclude the possibility that the appearance of the red-staining material in the blood vessels could be due to any displacement of glycogen, caused by the knife during the making of the sections. Before affirming finally that the migration of glycogen into the capillaries takes place only in cases where hyperglycogenolysis is occurring, as for instance in piqûre, it will be necessary to examine, by the above methods, a greater number of normal rabbits.

Some new apparatus. D. E. JACKSON.

Pathéscope films used to illustrate physiological demonstrations to students. ALEXANDER FORBES.

Photographs representing the growth of chickens fed with definite mixture of food-stuffs under laboratory conditions which have heretofore not led to success. THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

A convenient form of non-polarizable electrode for class use. THEODORE HOUGH.

A method of recording fundamental heart sounds directly from the heart. CARL J. WIGGERS and A. DEAN, JR.

Exhibit of photographically recording apparatus for studying the dynamics of the circulation. CARL J. WIGGERS.

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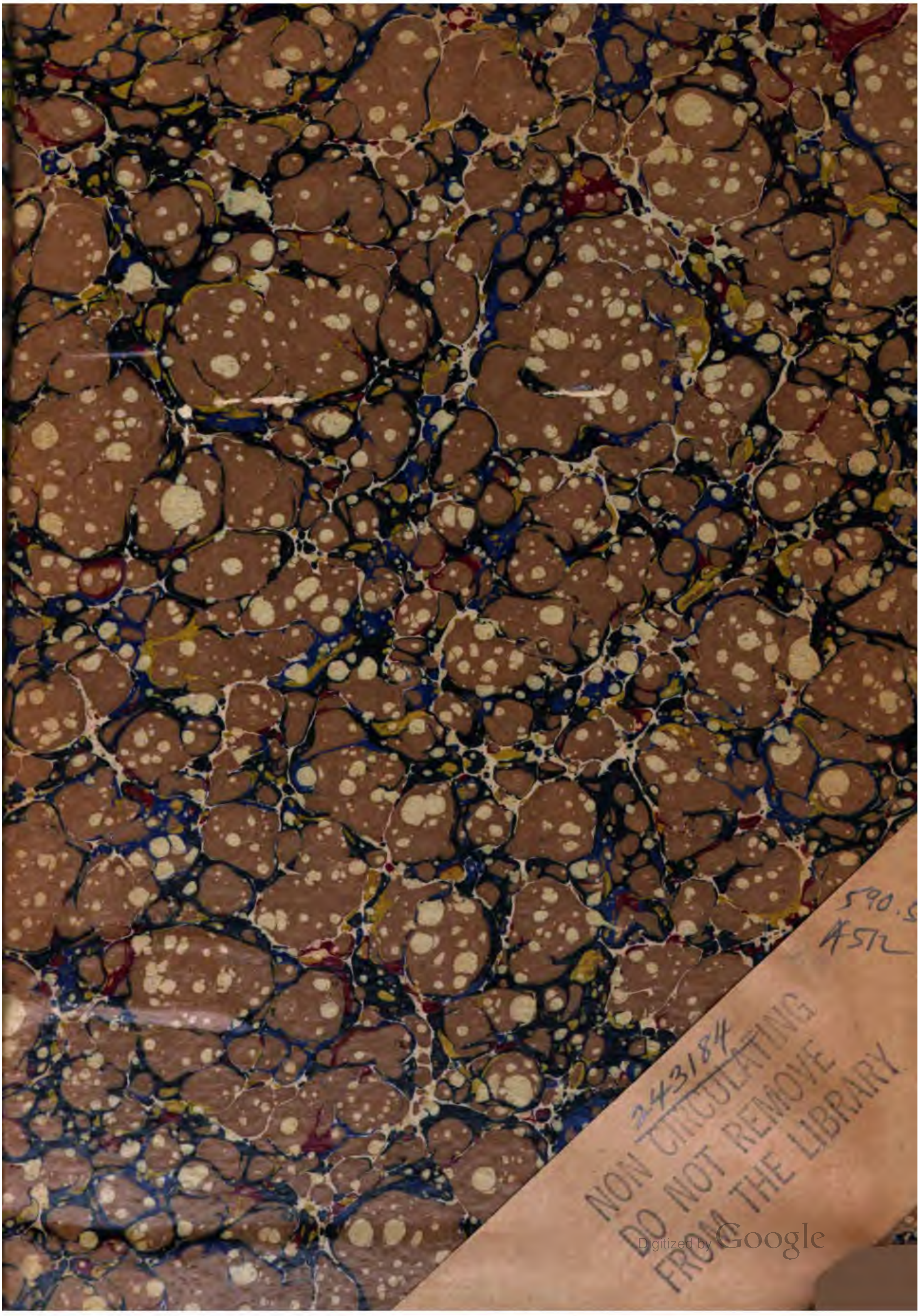

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